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7362 P*

Experimental Bronchogenic Pyogenic Lung Abscess.

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In a previous communication[1] a method was described for producing bronchogenic lung abscess with human tubercle B. The present report is on the production of bronchogenic lung abscess in dogs by the same method, using pyogenic organisms.

Thirteen dogs were narcotized with morphine sulphate and divided into 2 groups. In each of the first 7 dogs, 0.25 cc. of a suspension of a mixture of *Streptococcus hemolyticus* and *Staphylococcus aureus* in lipiodol was deposited in the peripheral portion of the right lower lobe. In 2 of this group a similar amount was also placed in the left lower lobe. This was accomplished by means of a ureteral catheter introduced through a bronchoscope. The procedure was carried out under fluoroscopic control, thus the deposit could be made at any desired location. In each of the second 6 dogs 0.25 cc. of a broth suspension of *Streptococcus hemolyticus* and *Staphylococcus aureus* was deposited in the peripheral portion of the right lower lobe. A similar amount of lipiodol was placed in the peripheral portion of the left lower lobe.

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* P represents a preliminary, C a complete manuscript.

[1] Adams, W. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 483.

Results. Group I. Three dogs were sacrificed on the 12th post-infected day. All presented indurated areas 1.5 to 2.5 cm. in diameter, one of which contained a cavity 0.33 cm. in diameter, and another a cavity of 1.0 cm. in diameter, each with a necrotic lining. The results in the other dogs are summarized in Table I. *Staphylococcus aureus* was cultured from one of the 2 cavities in animal 232.

TABLE I.
Bronchogenic Pyogenic Lung Abscess.

	Experiment			Results	
Dog	Lower lobe Involved	Material Injected (¼ cc.)	Days Duration	Indurated Area	Abscess Cavity
86	R.	Strep. and Staph. in lipiodol	12	2.5 cm.	1.0 cm.
93	R.	”	12	2.5 cm.	None
116	R.	”	12	1.5 cm.	0.33 cm.
167	R.	”	16	2.5 cm.	1.0 cm.
233	R.	”	16	None	None
	L.	”	16	None	None
232	L.	”	28	2.5 cm.	1.5 x 2.0 cm.
	R.	”	28	3.0 cm.	2.0 cm.
231	R.	”	28	2.5 cm.	1.0 cm.
244	R.	Strep. and Staph.	14	0.5 x 1.0 cm.	None
	L.	Lipiodol	14	None	None
254	R.	Strep. and Staph.	15	None	None
	L.	Lipiodol	15	None	None
281	R.	Strep. and Staph.	15	0.5 x 1.0 cm.	None
	L.	Lipiodol	15	0.5 x 1.0 cm.	None
237	R.	Strep. and Staph.	26	Very slight scarring	None
	L.	Lipiodol	26	Very slight scarring	None
243	R.	Strep. and Staph.	26	None	None
	L.	Lipiodol	26	None	None
248	R.	Strep. and Staph.	26	None	None
	L.	Lipiodol	26	None	None

Group II. One dog was sacrificed on the 14th post-infected day, 2 on the 15th and 3 on the 26th day. All lobes in which lipiodol had been deposited appeared normal except in one of the 15-day dogs. Here there was a slightly indurated area 0.5 x 1.0 cm. in dimension. All lobes in which micro-organisms had been placed appeared normal except the 14-day animal and one of the two 15-day dogs. In each of these a small indurated area 0.5 cm. in diameter was found, containing no cavity. In the 14-day animal, indurated areas were also noted in the right middle and right upper lobes. Microscopic sections through the indurated areas revealed infiltration with lymphocytes, endothelial cells and a few polymorphonuclear leucocytes.

The unsuccessful attempts to produce bronchogenic lung abscess in dogs[2, 3, 4] have been attributed to the efficiency of the cough reflex in the removal of foreign substances from the lungs. This factor was eliminated by maintenance of localization of the infective organisms with a heavy viscid media. Thus abscess formation was the usual result. It is not unlikely that maintenance of localization of pathogenic organisms occurs more readily in man than in dogs.

7363 P

Studies on Small Colony Variants of Staphylococcus Aureus.

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Small colony variants of bacteria are regarded by some investigators as a filterable stage in a life cycle of the organisms. Other workers do not feel that there is sufficient evidence in support of this theory. Hadley[1] has been one of the chief exponents of the idea that bacterial variation represents a cyclical development of the organisms and in this connection he described a small colony filterable form of the Shiga dysentery bacillus and other bacteria.

In the present investigation small colony variants of *Staphylococcus aureus* were studied with respect to their occurrence, morphology, physiological and antigenic properties and filterability. The variants were obtained by plating ageing broth or agar cultures and from cultures in lithium chloride broth. Their occurrence was irregular and rare and no means were found by which this type of variation could be induced. Tiny colonies were not observed at any time in very young cultures except occasionally in broth containing lithium chloride. They appeared only after the cultures had aged for a week or two and were more numerous after 3 or 4 weeks.

Well-isolated colonies of *Staph. aureus* are usually from 1 to 2 mm. in diameter when grown on nutrient agar. Those of the small colony variants could scarcely be discerned without the aid of a microscope. In the various strains they ranged from .04 mm., the smallest ever observed, to 0.5 mm., but most frequently they were

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[2] Cutler, E. C., and Schlueter, S. A., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 266.

[3] Schlueter, S. A., and Weidlein, I. N., *Arch. Surg.*, 1927, **14**, 457.

[4] Allen, D. S., *Arch. Surg.*, 1928, **16**, 179.

[1] Hadley, P., Delves, E., and Klimek, J., *J. Infect. Dis.*, 1931, **48**, 1.

about 0.1 mm. in diameter. The small colony variants had lost the power of pigment production, the growth on agar slants being white and translucent.

Morphologically some of the variants were not strikingly different from the original strains. In other cultures, however, the cells were very irregular in size. Some were in tetrads, others in pairs or single. Within each cluster of cells there were marked contrasts in size, some being much larger and others smaller than those of normal cultures.

Reversion to the normal* form occurred occasionally on agar. Sometimes in transferring from a stock culture of a variant strain, one or two large colonies with deep golden pigment appeared on the new slant and stood out sharply against the background of very small colonies which were scarcely visible. If the cultures were inoculated into nutrient broth, reversion to the normal took place more completely. Three strains never gave rise to normal colonies although many attempts were made to induce such a reversion.

These variants were very susceptible to changes in the composition of the media upon which they were grown. Growth could be stimulated in some strains by the use of veal infusion and dextrose and in others by the presence of blood in the medium.

The small colony forms were characterized by a change of physiological properties such as loss of fermentative powers. They all attacked dextrose, producing a definitely acid reaction in 48 hours. Lactose was not fermented and actually seemed to be inhibitory to their growth. The reactions in sucrose were similar to those in lactose. The original staphylococcus strains all attacked dextrose, lactose and sucrose, and readily produced a definite acid reaction in 24 hours.

One of the most striking characteristics of the small colony strains was their relatively slow rate of reproduction. This was shown by a comparison of the growth curves of a small colony variant and its parent strain in nutrient broth. At the end of 12 hours the normal bacteria had increased in numbers 40,000 times while the variants had multiplied to only 16 times the original number.

The variant organisms were avirulent for rabbits in the doses used although the normal strains from which they were derived showed a low degree of virulence.

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* The word "normal" is used in this paper as the most convenient term to designate the type of colony of *Staph. aureus* usually observed, which is round and smooth, having a diameter of 1 to 2 mm. on nutrient agar. It is recognized that this may not necessarily be the normal form.

Agglutination tests showed most of the variants to be antigenically similar to the normal strains from which they had been obtained.

All attempts to demonstrate a filterable stage in association either with the normal or the variant strains were unsuccessful. The organisms were grown in various media including Kendall's K medium and filtrations were carried out both with very young and old cultures. Growth could not be detected in any of the media, either liquid or solid, inoculated with filtrates from variant or from normal cultures.

It is suggested that these small colony forms occur as the result of injury to an occasional cell of the normal culture which results in a lowered rate of reproduction and the loss of several physiological properties.

7364 C

Ordinary and Mucoid Encapsulated Hemolytic Streptococci from Scarlet Fever.

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A series of strains of hemolytic streptococci and mucoid encapsulated streptococci was obtained from the throats of scarlet fever patients at Cook County Contagious Hospital.

Laboratory studies were made on a group of these strains to determine. (1) the results of their growth on chocolate agar, (2) their effect on the sugars sorbitol and trehalose and (3) the influence of mouse passage on capsule formation and colony characteristics.

Using chocolate agar Tunnicliff[1] found that hemolytic streptococci of scarlet fever produced no color change in this medium except occasionally a slight greening after several days' growth. The colonies almost always were conical in shape and slightly granular. Occasionally, however, a strain dissociated and produced atypical colonies that were large, moist, smooth and convex and turned chocolate agar green. These atypical colonies were not scarlatinal by the opsonic, agglutination or toxin neutralization tests.[2, 3]

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[1] Tunnicliff, R., *J. Am. Med. Assn.*, 1930, **94**, 1213.

[2] Tunnicliff, R., *J. Inf. Dis.*, 1931, **48**, 511.

[3] Tunnicliff, R., *J. Inf. Dis.*, 1931, **49**, 357.

With these investigations in mind, we studied the colony formation on chocolate agar of 62 strains of mucoid encapsulated streptococci and of 27 strains of ordinary hemolytic streptococci. Of the 62 strains of the mucoid type, 57 produced colonies resembling those considered typical by Tunnicliff. A second type of colony was noted in 34 of the 62 strains. This was large, distinctly white, glistening, smooth and convex and produced a greening of the surrounding media. Of the 27 strains of ordinary hemolytic streptococci, 23 produced the normal colony. In 18 the large, white green-color producing variant was noted. On ascitic fluid blood agar, no difference in colony formation could be noted.

A study of the toxin production of the variant was made to determine whether it possessed scarlatinal properties. From a satisfactorily stabilized colony of the variant of the mucoid strain, a 7-day broth filtrate was obtained. The toxin was diluted 1:1000 in sterile saline and 0.1 cc. was injected intradermally into 6 children known to be Dick positive. In 5 of the 6 children the filtrate from the variant produced a positive reaction. The Dick controls with commercial skin test scarlatinal toxin were all positive.

This emphasizes the results of an earlier investigation in which we reported that the specificity of the toxin of a scarlet fever streptococcus is retained not only when the strain becomes mucoid and encapsulated but also when it has completely lost its hemolytic property.[4]

In fermentation studies 26 of our mucoid strains were found to ferment trehalose but not sorbitol by using the Edwards[5] method of differentiation. This demonstrates a likeness in the reaction of the human mucoid type to the ordinary human hemolytic type. A group of 7 animal strains used for control purposes fermented sorbitol but not trehalose.

The final step in the study of the biological characteristics of these organisms was a consideration of the capsule formation and its relation to the type of colony produced on ascitic fluid blood agar pour plates.

Twenty-one ordinary hemolytic strains were studied for capsule formation by the India ink method. In 13 of these strains capsules of small size were demonstrable. Forty strains of saline washed organisms from 5 cc. broth cultures were injected into mice intraperitoneally. At death a loop of heart's blood was inoculated into 20% ascitic fluid infusion broth and into melted ascitic fluid blood agar tubes. After 18 to 24 hours the former cultures were examined for capsules and the pour plates were studied for colony formation. In 28 strains (70%) capsules could be demonstrated. Of the 8 strains in which capsules could not be identified before mouse inoculation, 7 showed them after this process. Mouse passage also increased the size of the capsules already present in a majority of cases.

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[4] Pilot, I., and Stocker, S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 181.

[5] Edwards, P. R., *J. Bact.*, 1932, **23**, 259.

Twenty-nine strains of the pour plates developed colonies of the mucoid type. Four plates were unreadable, 3 showed colonies of an intermediary type and 4 produced colonies unchanged from the original. In 3 of these 4 strains it was likewise impossible to demonstrate capsules in broth cultures.

7365 C

A Study of the Relation of Dietary Fats to Action of Thyroid Extract in Rats.

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A relation between dietary fats and thyroid activity has been reported by several investigators.[1-4] Jaffe[5] has reported interesting variations in the lipoid content of the thyroid in various diseases of the gland. Several reports have suggested the possibility that cod liver oil[1] or vitamins A and D in combination with iodine or iodized fat exert a favorable influence in Grave's disease.[6, 7, 8] These reports stimulated the author to test the effect of various oils including cod liver oil on rats rendered toxic by the administration of thyroid extract. The results are briefly reported here.

Methods. Both growing (3 months old and weighing from 100-110 gm.) and adult (150-195 gm.) rats were used in different groups of experiments. They were fed the standard Steenbock diet and kept in individual cages. One hundred mg. of thyroid extract

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[1] Mellanby, *J. Physiol.*, 1921, **55**, 7.

[2] McCarrison, *Ind. J. Med. Res.*, 1923, **11**, 1.

[3] Abelin and Kurosteiner, *Biochem. Z.*, 1928, **198**, 19.

[4] Abelin, *Biochem. Z.*, 1930, **228**, 88.

[5] Jaffe, *Arch. Path. and Lab. Med.*, 1928, **5**, 13; 1927, **3**, 955.

[6] Adamson and Cameron, *Canad. Med. Assn. J.*, 1928, **19**, 420.

[7] Fraser and Cameron, *Canad. Med. Assn. J.*, 1929, **21**, 153.

[8] Rabinowitch, *Canad. Med. Assn. J.*, 1929, **21**, 156.

(U.S.P.) was added to the diet. The control rats received the diet plus thyroid extract, water being used to make a paste. The treated rats received the diet plus thyroid extract plus the fat to be tested, the caloric intake being kept constant in the various series of experiments. Litter mates were distributed between the control and treated groups. The fats used were olive oil, cocoanut oil and cod liver oil. They were mixed with the diet to form a paste.

Thyroid extract plus olive oil. Group 1A—Adult rats. Twenty-four rats were used in this experiment. The 12 rats which received the olive oil (3 cc.) plus the thyroid extract lost weight less rapidly during the first 2 or 3 weeks than the controls, but after this preliminary period their rate of loss of weight was greater than that of the controls. In other words, the olive oil first inhibited and then facilitated the action of the thyroid extract. This 2-stage effect is shown in Fig. 1.

Growing rats. The preceding experiment was repeated in 24 growing rats, which are considered as being more resistant to thyroid extract (Falta.[9]) The control rats continued to grow for about 2 weeks and then began to lose weight.[9] The rats receiving olive oil at first grew more rapidly than the controls, then ceased growing and remained stationary for about one week, after which they lost weight more rapidly than the controls. Thus, the same 2-stage effect observed in the adult rats was also observed in the growing rats, the effect being more evident, however. (Fig. 2.)

We did not assay the olive oil for iodine and vitamin content.

Thyroid extract plus cocoanut oil. Group 1B. The experiment was repeated in 24 adult rats, using cocoanut oil (3 cc.). The addition of cocoanut oil to the diet very definitely augmented the action of thyroid extract from the start. The preliminary protective effect of olive oil was not observed. (Fig. 3.)

Thyroid extract plus cod liver oil. Group 1C. The experiment was repeated in 24 adult rats, using plain cod liver oil. The cod liver oil (3 cc.) in the dosage used contained approximately 0.03 mg. of iodine, 3,750 U.S.P. units of vitamin A, and 2,250 A.D.M.A. units of vitamin D. The cod liver oil had a more marked protective effect than olive oil. (Fig. 4.)

This experiment was repeated in 24 growing rats. In the experiment, the protective effect of cod liver oil was also more evident than that of olive oil.

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[9] Cameron and Carmichael, *J. Biochem.*, 1920, **45**, 69; Cameron and Sednak, *Am. J. Physiol.*, 1922, **58**, 7.

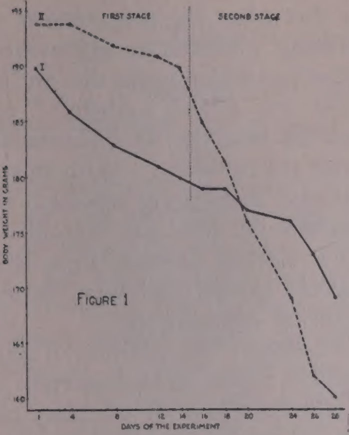

FIGURE 1

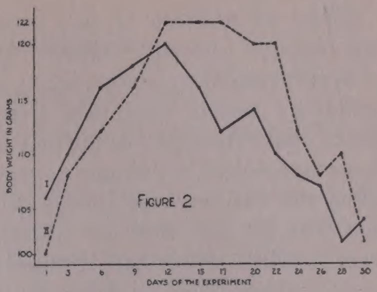

FIGURE 2

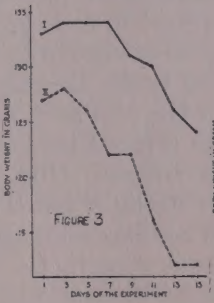

FIGURE 3

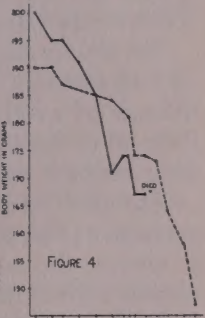

FIGURE 4

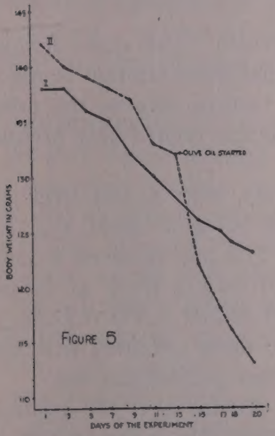

FIGURE 5

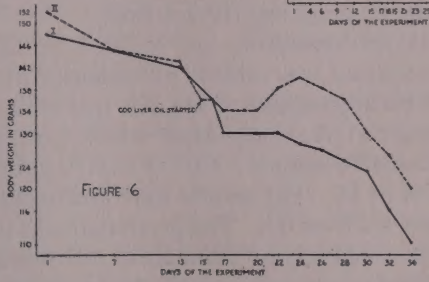

FIGURE 6

FIG. 1. Simultaneous feedings of thyroid extract and olive oil to adult rats, showing the 2 stages of olive oil action. Curve I is the weight curve of control rat receiving thyroid extract. Curve II is the curve (litter mates) of the rat receiving thyroid extract plus olive oil.

FIG. 2. Simultaneous feeding of thyroid extract and olive oil to growing rats (3 months old). Curve I is the weight curve of the thyroid fed control; II is the weight curve of the litter mate receiving thyroid plus olive oil.

FIG. 3. Simultaneous feeding of thyroid and cocoanut oil to adult rats. Curve I is the weight curve of the thyroid fed control; II of the litter mate receiving thyroid plus cocoanut oil.

FIG. 4. Simultaneous feeding of thyroid and cod liver oil to adult rats. Curve I is the weight curve of the thyroid fed control; II of the litter mate receiving thyroid plus cod liver oil.

FIG. 5. Effect of addition of olive oil to diet after the induction of hyperthyroidism. Curve I is the weight curve of the control; II of the litter mate receiving olive oil after the 13th day.

FIG. 6. Effect of addition of cod liver oil to diet after the induction of hyperthyroidism. Curve I is the weight curve of the control; II of the litter mate receiving cod liver oil after the 14th day.

Effect of addition of fats to the diet after the development of experimental hyperthyroidism. Mellanby[1] observed an aggravation of hyperthyroidism in man when butter was added to the diet and an inhibitory action when cod liver oil was added. Abelin[3, 4] observed that fats added to the diet after the induction of experimental hyperthyroidism in animals aggravated the condition. With this in mind, the various experiments performed above were repeated, except that the fats were not added to the diet until the thyroid extract administration had resulted in a marked decrease in weight, usually about 2 weeks after the thyroid extract had been started. Twelve rats were used in each group of experiments.

The results may be summarized as follows: The addition of olive oil and cocoanut oil to the diet caused a rather sudden increase in the rate of weight loss. (Fig. 5.) The addition of the plain cod liver oil to the diet arrested the loss of weight for about 10 days, after which it was resumed. (Fig. 6.)

The effect of a Vitamin A and D concentrate. This quite definite protective effect of cod liver oil suggested that some attempt be made to analyze the factors concerned. These factors may be the presence of iodized fat, or Vitamin A or D. In this regard only preliminary findings may be reported.

Hyperthyroidism was induced in 12 rats, then a cod liver oil concentrate was added, the quantity of fat being so small that no caloric adjustment of the diet was made. The rats received a daily dosage of the concentrate which contained only a trace of iodine (Kendall's method) 10,000 U.S.P. units of A and 3,300 A.D.M.A. units of D. The results were analogous to those obtained with the plain cod liver oil. The protection was neither greater nor less.

It would appear from these preliminary results as if the vitamin content of the cod liver oil played an important rôle in the protective action. It is well known that iodine alone does not protect against the action of thyroid extract. These results would support the clinical observations of Cameron and others[6, 7, 8] which indicate that in Grave's disease Vitamins A and D increase the therapeutic effectiveness of iodine. However, these studies should be pursued further to ascertain the effect of the various fats *per se*, of iodized fat, of Vitamin A, and of Vitamin D. Vitamins A and D should be studied particularly because of the presence of excessive calcium elimination and of liver damage in Grave's disease. The effect of lecithin or phospholipins should also be studied.

7366 P

Dissociation of the Gonococcus.

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Dissociation of the gonococcus has been implied in the work of Herzog[1] and Warden[2] as early as 1913. However, very recently two contemporary schools of thought have appeared in the literature; the French (represented by Ramsine,[3] Milochevich,[4] and Lavrinowicz[5]) and the German (represented by Kandyba[6] and Delbanco-Lorentz[7]) who are convinced that changes in the morphology of the gonococcus exist *in vitro* and *in vivo,* while to the contrary, the Italian school (represented by Porcelli,[8] Sechi,[9] and Debiasi[10]) firmly believes that such changes have very little in common with the gonococcus and are due to errors in technique and interpretation. In view of the conflicting literature the present report, as well as more detailed results to appear later, may prove significant.

Dissociation was attempted and accomplished with 4 stock strains of the gonococcus. During the entire study a modification of Hitchen's semi-solid medium was found satisfactory. Dissociation was produced by ageing and the use of immune serum.

Primary changes through ageing were noted when a sparsely inoculated ascitic agar plate was sealed and incubated for varying periods of time. These changes were evidenced as secondary or papillated colonies superimposed on the original colony or streak. Briefly, the sequence of events was as follows: The secondary growth was yellowish and opaque and when stained showed a bead-like bacillus (as though cocci were strung together to form a closely beaded chain). This form through further ageing and cultivation developed 2 types of growth, namely, a large opaque smooth colony, 3 mm. in diameter, composed of typical biscuit-shaped diplococci but taking the Gram counterstain very deeply and a small colony, 0.5 mm. in diameter, composed of tiny gram negative micrococci.

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[1] Herzog, H., *Virchow's Arch.*, 1913, **212**, 243.

[2] Warden, C. C., *J. Inf. Dis.*, 1913, **5**, 12.

[3] Ramsine and Milochevich, *Compt. rend. Soc. de Biol.*, 1928, **99**, 1261.

[4] Milochevich, *Compt. rend. Soc. de Biol.*, 1929, **100**, 70.

[5] Lavrinowicz, *Compt. rend. Soc. de Biol.*, 1925, **93**, 789.

[6] Kandyba, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1922, **96**, 347.

[7] Delbanco-Lorentz, *Dermat. Woschr.*, 1923, 1137.

[8] Porcelli, R., *Gior. ital. di Dermat. e Sifil.*, 1929, **70**, 1255.

[9] Sechi, *Gior. ital. di Dermat. e Sifil.*, 1931, **12**, 1045.

[10] Debiasi, *Clin. Ostet.*, Rome, 1930, **32**, 665.

The influence of immune serum was studied by making serial transplants through semi-solid agar containing immune serum in dilution of 1:50. In brief the results were as follows: Modifications in size were first noted in the fifth tube of the series. The cocci became greatly enlarged, took the counterstain more deeply, and in several instances the tube, next in series to the one composed of giant forms, contained a peculiar gossamer globoid body with tiny coccoid granules both within and without the cell. Most of the pleomorphic changes appeared in the seventh, eighth and ninth tubes of the series. At first the Gram negative coccus was accompanied by a Gram positive coccus, but in later tubes the Gram negative form disappeared, to be displaced by a variety of pleomorphic forms. The colony forms on ascitic agar included a grayish semi-translucent smooth colony with Gram negative diplococci, a gray opaque colony, consisting of giant cocci, and a yellowish opaque colony which contained either Gram positive cocci or granular rods or both cocci and rods. The yellowish opaque colony was the one which gave place to the greatest variety of forms on subculture; these included bacillo-coccoid forms, Gram positive diplococci, polar-body cocci, ring-forms (periphery staining dark and center light), beaded rods, and sometimes a peculiar sheath form which contained within it granules. Reversion to the original form was made possible through subculture in ordinary media occasionally through growth in immune serum, and occasionally by use of cystic or pericardial fluid.

During the course of this study, 3 distinct colony types were noted: Type I, the typical form, represented by a translucent grayish-green, slightly granular to smooth colony, 1 to 2 mm. in diameter, staining as a Gram negative diplococcus, and fermenting dextrose; Type II, represented by a large grayish to white opaque and hardier colony, 1.5 to 3.0 mm. in diameter, staining as a Gram positive or polar body diplococcus, and fermenting dextrose, maltose, sucrose, levulose, and occasionally lactose and other sugars; Type III, represented by a tiny colony (similar to Hadley's "G" colonies), 0.5 to 0.8 mm. in diameter, very rough, flat and adherent to the surface of the agar, growing sparsely, and consisting of a fusiform Gram negative granular bacillus (2.0 to 2.5 micron in length) and fermenting no sugars. Between these were interspersed other forms, some with chromogenic properties and varying in morphology such as beaded rods, dumbbell shapes, polar body diplococci, ring forms, globoid phantom forms, and tiny Gram negative micrococci. The pleomorphic forms and particularly the bacillary forms were least stable, while the Gram positive diplococcus and the large opaque colony form was most stable.

In regard to the fermentation of sugars, it was noted that the typical Gram negative diplococcus fermented dextrose; the extreme "R" or tiny colony form failed to ferment sugars, but as dissociation progressed, first dextrose alone, and then maltose, sucrose, levulose and lactose were utilized. The Gram negative micrococcus failed to ferment carbohydrates, but when the typical form was attained fermented dextrose. Therefore, reversion was accompanied by return to fermentation type.

The agglutination for the homologous and typical cyclostage attained a titer of 1:5120, while the titer for the opaque form (polar-body and Gram positive diplococcus) was 1:160 to 1:320. It was difficult to obtain a homogeneous suspension for the "R" form or the tiny Gram negative micrococcus, so that no determinations were made for these stages.

7367 C

Accumulation of Bacteriophage in Spleen and Liver Following Its Intravenous Inoculation.

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The rôle of the reticulo-endothelial system in removing microorganisms from the blood stream has been generally recognized in recent years. The earlier work of Bull[1] in this field has been supported and extended by others, particularly Cannon[2, 3] and his coworkers. The question arose as to whether or not a readily filterable biological principle such as bacteriophage, which is probably particulate and of the general order of magnitude of the filterable viruses, would be removed from the blood stream by organs of the reticulo-endothelial system to a greater degree than by organs with few fixed phagocytic cells.

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[1] Bull, Carroll G., *J. Exp. Med.*, 1915, **22**, 475, 484.

[2] Cannon, Paul R., Sullivan, F. L., and Neckermann, E. F., *J. Exp. Med.*, 1932, **55**, 121.

[3] Sullivan, F. L., Neckermann, E. F., and Cannon, Paul R., *J. Immunol.*, 1934, **26**, 49.

Y. Shitate[4] reported on the fate of bacteriophage in the body. Since he made no attempt to obtain quantitative results, his findings do not aid in answering the above question. Smirnow and Goldin[5] made a similar investigation on the fate of phage in the guinea pig's body after inoculation into the blood stream. Unfortunately they selected a 24-hour period for their first examination of the organs. Phage was found in low concentrations in blood, liver, spleen and kidneys. Sullivan, Neckermann and Cannon[3] have recently pointed out that although bacteria are present in much greater numbers in the spleen than in the blood 1 hour after an intravenous inoculation, that organ may be sterile in 24 hours. This may explain why Smirnow and Goldin failed to find any significant difference in bacteriophage titer between the blood and the reticulo-endothelial organs after so long a period as 24 hours.

Method. 15 albino rats (150-200 gm.) were etherized and injected in the exposed femoral vein with 2 cc. of staphylococcus phage. (1 cc. was injected in rats 11, 12 and 13.) In the case of rats 12-15 0.5 cc. of blood was collected from the clipped tail within 5 minutes. Two hours later the animals were again anesthetized and 0.5 to 1.0 gm. of various tissues were removed, ground with sand and 5 cc. of saline in a test tube. This material was diluted in steps of 1-10 with plain nutrient broth. A fresh sterile pipette was used for each dilution. One drop of a broth culture of the susceptible organism was seeded into each tube. Inhibition of growth was noted after 48 hours' incubation. Occasional doubt as to inhibition due to resistant secondary growth was removed by subculturing from the doubtful tube to an agar slant and noting the presence or absence of lytic areas. An additional procedure, the plaque enumeration test, was used in the case of rats 11-15. 0.1 cc. of material from the undiluted ground-up material or an appropriate dilution, as experience suggested, was placed on the surface of an agar plate with 0.1 cc. of a heavy suspension of staphylococcus. (5.0 cc. saline to an agar slant.) This was carefully spread with a bent glass rod. The number of plaques per plate was estimated after 24 hours' incubation.

Ten rats were injected in a similar manner with a saline suspension of staphylococcus. After 2 hours the animals were killed and the number of viable bacteria in the various tissues was estimated by using the serial dilution method described above, noting the highest dilution showing growth after incubation. A plate count was made on material from rats 6-10.

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[4] Shitate, Y., *Orient. J. Dis. Infants,* 1931, **10**, 1.

[5] Smirnow, P., and Goldin, M., *Cent. f. Bak.,* Orig. I, 1931, **122**, 512.

TABLE I.

Titration of Phage in Inoculum and Tissues.

	Final Tube to Show Evidence of Phage in Decimal Dilution Series.							
Rat		5 min.	2 hr.					
No.	Inoculum	Blood	Blood	Spleen	Liver	Brain	Kidney	Muscle
1	8		4	5	6			0
2	9		3	6	5			0
3	9		4	4	5			2
4			4	6	6			0
5	9		5	6	5			3
6	9		4	6	5			3
7	8		4	6	6			2
8	9		3	3	6			0
9	9		4	5	5			0
10	8		4	5	5			0
11	10		4	5	5	1	2	
12	10	6	2	5	4	2	3	
13	10	6	1	5	5	0	3	
14	10	7	2	6	4	2	4	
15	9	7	2	6	5	2	3	
Mean	9.0	6.5	3.3	5.3	4.9	1.4	3.0	1.0
	Phage units per gm. as determined by plaque numeration on 0.01 gm. or less of sample.							
11	100 mil.		200	20,000	2,500	0	300	
12	1000 mil.	135,000	0	54,000	1,000	0	20	
13	100 mil.	40,000	0	3,600	30,000	0	20	
14	100 mil.	2,400	0	60,000	300	0	50	
15	1000 mil.	600,000	0	2,400	240	1	20	
Mean	460 mil.	194,350	40	28,000	6,800	0	82	

Results. Table I shows the results obtained in the phage experiment involving 15 rats. The mean of the values obtained by the inhibition test indicates that phage was present in the spleen 2 hours after injection in approximately* 100 times the concentration found in the blood. The liver contained about 40 times as much as the blood, while the kidney titer was about the same as that of the blood. The brain and skeletal muscle yielded only about 1/100 as much phage as the blood. Blood collected immediately after injection contained over 10 times as much phage as did the spleen at 2 hours. The results obtained by the plaque method indicated that the spleen contained approximately 700 times and the liver 170 times as much phage as the blood at the period examined. Yet immediately after injection the blood contained around 7 times as much phage as did the spleen at 2 hours.

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* Inasmuch as a difference of 1 tube in the decimal dilution series employed represents a tenfold difference in phage content, the antilogarith (base 10) of the number of the final tube showing phage gives the numerical representation of titer.

The fate of microscopic particles including bacteria injected intravenously has been well established by other workers. Nevertheless the results of a set of such experiments on the fate of intravenously injected staphylococci are recorded in Table II. These data, obtained under conditions similar to those existing in the phage experiments, are therefore better adapted for comparison with the latter results. By the dilution method the spleen contained 300 times as many bacteria as did the blood 2 hours after injection. The liver contained about 100 times as many, while the brain contained about the same number as the blood. Plating methods indicated that the spleen contained approximately 1600 times and the liver 1000 times as many bacteria as were found in the blood. The brain and blood had about the same number by this method. It is to be noted that there is a general similarity between these results and those obtained with bacteriophage.

TABLE II.
Titration of Staphylococcus in Inoculum and Tissues.

Rat No.	Final Tube to Show Growth in Decimal Dilution Series.				
			2 hr.		
	Inoculum	Blood	Spleen	Liver	Brain
1	8	3	4	5	3
2	7	4	6	6	4
3	6	4	5	4	4
4	5	4	5	5	4
5	5	3	5	4	3
6	9	4	6	6	4
7	8	4	5	6	3
8	8	4	7	8	4
9	7	3	6	5	3
10	7	3	7	6	3
Mean	7.9	3.6	6.1	5.5	3.5
	Organism per gram as determined by plating 0.01 gm. or less of sample.				
6	36 mil.	0	80,000	60,000	0
7	100 mil.	50	22,000	16,400	0
8	100 mil.	50	60,000	14,000	50
9	30 mil.	0	1,080	1,320	0
10	30 mil.	0	720	1,200	0
Mean	59 mil.	20	32,760	18,584	10

The plaque method for phage and the plating method for bacteria gave more striking results than the serial decimal dilution methods. In the phage experiments plaques were seen on but one plate streaked with blood drawn at 2 hours. On the other hand it was often necessary to use dilutions of the splenic substance to avoid complete lysis on the plate.

Significance. Aside from any direct bearing on the fate of bacteriophage in the body these results raise several questions of somewhat wider interest. Does the mechanism of removal of phage units from the blood stream by the spleen and liver or by the circulating phagocytes as reported by Eliava[6] involve the mechanism of phagocytosis as has been observed for microscopic particles? A direct answer to this question is desirable but how to obtain such evidence does not appear obvious. The second question is concerned with the possible existence of an analogy between the fate of phage in the blood stream and that of filterable viruses which may get into the blood of the host. An answer to this query would be of value in a better understanding of those diseases caused by the filterable viruses.

Summary. It has been demonstrated that bacteriophage injected intravenously into rats accumulates in the spleen and liver. These organs accumulate phage to the same general degree as they do bacteria.

7368

Effect of Acidosis upon Production of Agglutinins and on Blood Proteins in Rabbits.

W. W. BRANDES. (Introduced by J. P. Simonds.)

From Baylor University College of Medicine, Dallas, Texas.

A few reports in the literature indicate that acidosis hinders the production of agglutinins. Various substances have been used to produce an acidosis. Ammonium chloride produces a prolonged acidosis in man.[1, 2] Davesne and Haber[3] thought they obtained a decrease in agglutinin production while administering hydrochloric acid. Moen and Reimann[4] obtained a poor response or no agglutinin production to typhoid vaccine in uncontrolled diabetic patients with acidosis.

In the experiments reported here, rabbits were given three 0.5 cc. intravenous injections of suspensions of killed typhoid bacilli on alternate days. In 12 normal rabbits the agglutinin titer averaged

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[6] Eliava, G., *Comp. rend. Soc. de Biol.*, 1930, **105**, 829.

[1] Burdon, J. B., and Haldane, S. H., *J. Physiol.*, 1921, **55**, 265.

[2] Lui, S. H., and Hastings, A. B., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 781.

[3] Davesne, J., et Haber, P., *Annales de l'Institute Pasteur*, 1932, **49**, 220.

[4] Moen, J. K., and Reimann, H. A., *Arch. Int. Med.*, 1933, **51**, 789.

1:320 at the end of the first week, with variations from 1:60 to 1:5120 dilutions, 1:1450 at the end of the second week, 1:800 third and 1:750 at the end of the fourth week.

Another series of 12 rabbits were given, in addition, 0.3 to 1.3 cc. of a 10% solution of ammonium chloride intravenously daily. The average agglutinin titer at the end of the first week was 1:50 with variations of from 0 to 1:320, second week 1:20, third week 1:10 and at the end of the fourth week 1:10 dilution. Three rabbits died during the first and second week. The animal with a titer of 1:320 at the end of the first week, later dropping to 1:10, had a plasma CO_2 combining power of 46 volumes %, which progressively decreased later to 23.0 volumes %. In 3 rabbits with no agglutinin production throughout the plasma CO_2 was consistently below 33 volumes %.

The average CO_2 values in the animals receiving ammonium chloride was 27 volumes % with variations from 46 to 18.3 volumes %. The average plasma CO_2 combining power in normal rabbits was 50 volumes %.

The plasma proteins in the first series increased, especially the globulin fraction which had increased 22% over the initial value. In 6 rabbits receiving ammonium chloride changes in blood protein were much less marked. At the end of the third week the globulin fraction was slightly increased, however, the red cell counts also increased and the animals lost weight during the course of the experiments, partly due at least to dehydration. Repeated bleedings are said to cause an increase in blood proteins.[5] Three rabbits bled 6, 7 and 17 times, from 1.5 to 2.5 cc. at each bleeding, showed an increase in globulins of 15, 17 and 40% in a period of 3 weeks. Three animals given suspensions of graphite intravenously also had increased globulins of 8, 35 and 45%.

The above results indicate that acidosis diminishes the production of agglutinins to a marked extent. Stimulation of the reticulo-endothelial system by various means, such as by the injections of foreign proteins, repeated hemorrhages or particulate matter apparently produces an increase in plasma proteins, particularly the globulin fraction. Parallel blood volume changes should be made, however, to draw definite conclusions on this phase.

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[5] Schmidt, R. A., and Tuljschinskaja, K., *Z. f. Immunit. u. Exp. Ther.*, 1931, **70**, 8.

Minnesota Section.

University of Minnesota, April 18, 1934.

7369 P

Effects of Various Salts on Carbohydrate Metabolism and Blood Pressure in Diabetic Children.

W. H. THOMPSON AND IRVINE McQUARRIE.

From the Department of Pediatrics, University of Minnesota.

The excessive loss of minerals from the body in diabetic acidosis has recently been presented quantitatively in detailed balance studies by Peters[1] and Atchley[2] and their co-workers. Kydd[3] has emphasized the depletion of chloride in excess of other electrolytes, as if the base lost with the ketone acids might be derived from NaCl rather than from bicarbonate. He stresses the need for replacement of chloride during recovery from coma. Diabetic children not infrequently manifest an unusual craving for salt, even when not excreting ketone bodies in abnormal amounts. This has been interpreted by Joslin as most likely being due to the high potassium content of their vegetable diets.

The present study was suggested by our experience in the case of a 15-year-old boy, who, as he emerged from diabetic coma, begged for salt and voraciously consumed 80 gm. of NaCl in the course of 24 hours. The water intake was proportionately increased. When it was observed that the craving for salt did not abate long after the disappearance of ketosis and that the patient continued to take 60 gm. daily before being satisfied, he was placed in the metabolism ward where complete balance studies could be carried out under standard conditions. He was given a constant diet containing protein 16, fat 33, carbohydrate 42, and water 1200 gm. every 6 hours.

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[1] Peters, J. P., Kydd, D. M., Eisenman, A. J., and Hald, P. M., *J. Clin. Invest.*, 1933, **12**, 377.

[2] Atchley, D. W., Loeb, R. F., Richards, D. W., Jr., Benedict, E. M., Driscoll, M. E., *J. Clin. Invest.*, 1933, **12**, 297.

[3] Kydd, D. M., *J. Clin. Invest.*, 1933, **12**, 1169.

This was made up from powdered milk, bread, butter, eggs, cane sugar, lemon juice and a small amount of powdered yeast. The insulin dosage was kept constant for each period, totaling 32 units per day. This dosage of insulin permitted the occurrence of glycosuria in every 6-hour period, thus making comparative measurements possible.

Shortly after the study was undertaken, it was discovered (1) that the high NaCl intake was accompanied by an elevation of arterial blood pressure from an average of 110 mm. of mercury systolic and 80 diastolic to 175 systolic and 115 diastolic and (2) that the urinary excretion of glucose fell from a control level of between 60 and 70 gm. to between 10 and 20 gm. daily under the same conditions. Reduction of the NaCl intake to the control value of about 4 gm. daily caused a return of the glucose excretion to the original level and a fall in the blood pressure to normal. Ketosis was never present. Within a period of 3 months these effects have been reproduced 4 times in this patient. The changes in sugar output and blood pressure regularly took place in from 24 to 48 hours. Similar results as regards both glucose excretion and blood pressure changes have since been obtained under almost identical conditions in 2 other cases of moderately severe diabetes in which the salt-craving factor was much less prominent.

The effect of the high NaCl intake on the glucose tolerance curve was to lower the fasting and peak values without greatly shortening the period required for return to the fasting level.

In an attempt to determine whether either the Cl ion or the Na ion alone, rather than their combination, was responsible for the changes observed, the effects of sodium bicarbonate, sodium citrate and KCl were compared with those of similar amounts of NaCl. While the 2 other sodium salts had similar though less marked effects than NaCl, the potassium salt had an opposite action in the one case in which it was given at the rate of 5 gm. every 6 hours. In this instance the sugar excretion per 24 hours rose to nearly double that of the control period, while the systolic blood pressure fell to a level approximately 20 mm. of mercury below that of the control period. Both of these effects disappeared within 24 hours after withdrawal of the KCl.

These observations gain in interest when considered in the light of recent discoveries concerning the relationship of the hypophysis, the diencephalon, and the adrenal glands to sodium chloride balance, carbohydrate metabolism and blood pressure control. A single experiment on the effect of adrenal cortical hormone in large doses

(8 cc. of eschatin per day for 3 days), however, gave only negative results.

In the case of one normal control subject the ingestion of 15 gm. of NaCl every 6 hours caused an increase in blood pressure from 115/80 to 148/100 mm. of mercury, when the subject was on the simple, relatively-low-potassium diet given to the 3 diabetic children, but little or no change when he was taking an ordinary mixed diet containing liberal amounts of vegetables and meats. The possible effect on the carbohydrate metabolism of this subject was not determined.

Further studies are being made on (1) the specific effects of the different ions, (2) changes in blood volume with variations in salt intake and (3) effects, if any, of salt ingestion on the respiratory quotient.

7370 P

Effects of Radiation on Tissue Cultures of Lymph Node.

WILHELM STENSTROM AND JOSEPH T. KING.

From the Cancer Institute and the Department of Physiology, University of Minnesota.

Cultures of the mesenteric lymph node of adult rabbits were exposed to the type of radiation usually used for treatment of lymph nodes. The high voltage therapy machine at the University Hospital was utilized.

The water cooled X-ray tube was run with a current of 30 milliamperes at 200 K.V. A focus-surface distance of 50 cm. was used. The filter used in the preliminary experiments consisted of ½ mm. copper plus 4.6 mm. aluminum (the aluminum in the ionization chamber).

The preliminary experiments consisted of cultures radiated with 1, 2, 4 and 8 erythema doses (800, 1600, 3200 and 6400 roentgens). It became evident that higher doses were required to get the effects desired on the most resistant cells present.

The distance could not conveniently be reduced since it was desired to keep the cultures in a thermostat. In order to cut down the time of exposure the copper was removed from the filter, leaving the 4.6 mm. aluminum. The radiation was continued for 145 min. The absorption in the glass vessels was about 5%. With this taken into account the dose reaching the cultures is about 19 erythema doses or 15,000 roentgens, given at rate of 103 roentgens per min. The half value layer was found to be 0.395 mm. of copper. Under these conditions the radiation is quite inhomogeneous but quite comparable to that used during treatment of patients.

The data discussed here were derived from cultures radiated at this higher dose. The discussion is limited to the cells migrating from the explant as seen in the living preparation.

Cultures were radiated at 37.0°C. Maximow slides and Carrel flasks were used. The culture medium was composed of 1 part heparinized plasma and 2 to 3 parts Tyrode extract of 6-day chick embryo. The study covers a period from immediately after radiation to 32 days. Controls were kept in the thermostat while the radiation was in progress. They were protected with lead.

The rapid dense migration of lymphoid cells seen around the controls is practically abolished at this dose. Usually a narrow rim composed of a small number of cells is seen in the radiated cultures. These cells are usually not ameboid when seen at the end of the radiation. Presumably they migrated early in the radiation period. Rarely there are some cells that show ameboid form for a few hours. These are usually the larger forms. The migration zone does not widen appreciably after the radiation period.

The large reticular macrophages appear at some variable later time in both experimental and control preparations. In the living preparation there is no striking difference early. At about 48 hours these cells become less ameboid and cease migration in the radiated cultures. They are not killed. They do not proliferate. In the controls migration and proliferation continue. In the later stages many giant cells form.

The fibrocytes are least influenced by the treatment. The response is variable. In some cultures luxuriant growth takes place, in others practically none. The cultures showing good growth may not show any qualitative change. Those showing marked inhibition also show, in many instances, unusual cells. Cells may show excessive branching and abnormal shapes. Cells may be found in unusual arrangement. In many there are multiple nuclei. Many cultures show micro forms.

The observations made on the living cultures suggest that these clearly defined cell families show marked differences in sensitivity to radiation under the conditions of the experiment.

The fixed and stained material will be described in the final publication.

7371 P

Metabolism Studies with Rats Suffering from Fat Deficiency.*

GEORGE O. BURR AND A. J. BEBER

From the Botany Department, University of Minnesota.

Wesson and Burr[1] showed that rats reared on the fat-deficient diet of Burr and Burr[2] gave respiratory quotients above unity and exceptionally high metabolic rates after a carbohydrate meal. Normal rats had lower basal rates and did not show the very high respiratory quotients or great specific dynamic action of the food.

This work has been continued over a period of 2 years with selected rats and their litter mate controls. A different technique has been used in order to follow gas exchange of the rats under normal conditions over long periods of time. An open-circuit apparatus with constant air stream has been used in conjunction with an automatic sampler (to be described elsewhere) which collects the dry samples over mercury and holds them for analysis. The Haldane-Carpenter[3] apparatus was used for determining the CO_2 and O_2.

The rats have been divided into 3 groups: those reared on stock diet; those reared on fat-deficient diet; and those reared on fat-deficient diet and then cured with certain fats. Three different methods of study have been applied. Some rats were starved 16 hours and then 2 periods of starvation metabolism were taken, followed by 4 periods while eating the normal diet. Other rats were run for 24 hours on the normal diet, then the food was removed and the behavior during 24 hours of starvation was followed. Still other rats were kept in the metabolism cage 48 hours on the normal diet. The periods were 4 hours long so that 6 samples were required for a 24-hour run. All runs were made at 28.5-29.0° C. The colony temperature is kept at 24-26° C. at all times.

The results show clearly that fat-deficient rats are very different from stock animals and that fat-deficient rats which have been cured with small doses of fats return to a much more nearly normal gas exchange. The most marked differences shown by the fat-

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*Supported by grants from the Medical Research Fund and General Research Fund of the University of Minnesota and from the National Live Stock and Meat Board.

[1] Wesson, L. G., and Burr, G. O., *J. Biol. Chem.*, 1931, **91**, 525.

[2] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

[3] Carpenter, T. M., Fox, E. L., and Sereque, A. F., *J. Biol. Chem.*, 1929, **83**, 211.

deficient rats are higher basal rate, higher specific dynamic action of food, and higher respiratory quotients. These results are of especial interest since the runs were made over long periods of time under normal conditions. The respiratory quotients of the fat-deficient rats remain above unity for as long as 12 hours out of 24. They, therefore, synthesize every day large amounts of fat, but this synthetic fat does not prevent the fat deficiency.

Pacific Coast Section.

Mount Zion Hospital, San Francisco, April 18, 1934.

7372 P

Effect of Pilocarpine, Atropine, Morphine, and Epinephrine upon Oral and Pharyngeal Mucous Secretion.*

MARY F. MONTGOMERY. (Introduced by C. D. Leake.)

From the Department of Surgery, University of California Medical School, San Francisco.

When Schaefer and Moore[1] gave pilocarpine to dogs without parotid and submaxillary glands, they observed the accumulation of a small quantity of exceedingly viscid mucus which appeared to be formed by the oral mucous glands. Langley,[2] in referring to the effects of suprarenal extracts found that they caused "a lively secretion from all the salivary glands and some secretion from the buccal, esophageal, and tracheal mucous glands."

During the course of experiments upon the oral and pharyngeal mucous membranes, I observed the effect of the following drugs upon their secretory activity: pilocarpine, atropine, morphine, and epinephrine.

1. *Pilocarpine sulfate.* In Table I are listed the quantities of mucous secretion which I obtained at 5 minute intervals after intravenous injections of 0.2, 0.3, and 0.5 mg. per kg. of pilocarpine in 3 dogs each weighing 10 kilos. The salivary and orbital glands had been extirpated and the remaining secretion was collected from an esophageal fistula. A total of 35 experiments were performed upon these dogs. Pilocarpine injections result in the production of surprisingly large quantities of mucous secretion.

2. *Atropine sulfate.* Atropine in sufficient dosage abolished the reflex secretion of the mucous glands and their response to pilocarpine as well.

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* This work has been conducted under a grant from the Christine Breon Fund for Medical Research in the University of California Medical School.

[1] Schaefer, E. A., and Moore, B., *J. Physiol.*, 1895, **19**, xiii.

[2] Langley, J. N., *J. Physiol.*, 1901, **27**, 237.

TABLE I.

Grams of Mucous Secretion Elicited by Intravenous Pilocarpine Injection.

Min. after injection	Dose 0.2 mg./kg. — Dog No. 4	8	11	Dose 0.3 mg./kg. — Dog No. 8	11	Dose 0.5 mg./kg. — Dog No. 11
5	3.6 gm.	1.7 gm.	2.9 gm.	5.5 gm.	3.9 gm.	7.0 gm.
10	1.2	1.3	1.5	2.8	1.9	3.7
15	1.3	1.4	1.1	1.7	2.2	2.9
20	1.5	1.5	1.4	1.7	1.3	2.0
25	1.5	1.3	1.3	1.7	1.6	2.7
30	1.2	1.2	1.5	1.4	1.4	1.9
35	0.9	0.9	1.3	1.4	1.3	2.4
40	1.2	0.9	1.1	1.0	1.1	1.4
45	1.2	0.9	1.0	0.9	1.1	1.1
50	1.1	0.6	0.7	0.7	0.9	1.4
55	0.9	0.4	0.6	0.6	0.9	1.2
60	0.6	0.5	0.6	0.4	0.9	0.8
Average of	3 exp.	3 exp.	2 exp.	5 exp.	3 exp.	2 exp.
Normal 5 min. collections	0.2-0.8 gm.	0.1-0.5 gm.	0.15-0.45 gm.			

3. *Morphine sulfate.* The subcutaneous injection of 15 mg. of morphine produced, in the dogs described above, a transitory augmentation of the mucous secretion coinciding in time with the licking and retching movements and lasting 5 to 10 minutes. During the succeeding period of depression the secretion was normal or subnormal in quantity.

4. *Epinephrine.* The effect of intravenous injection of epinephrine was observed directly in the mouth only. The 4 dogs used were given enough sodium amytal intravenously to produce relaxation of the jaws (30 to 40 mg. per kg.). Repeated experiments were performed on each animal. The response of 2 of the dogs to epinephrine was determined under morphine and ether anesthesia as well, and was found not to differ from their response while under sodium amytal anesthesia. The dosage of epinephrine was 0.25 to 1.0 cc. of a 1/10,000 solution injected intravenously, but the size of the dose had no apparent influence upon the quantity of secretion produced. Neither did the repetition of the injection from 2 to 5 times at one to 5 minute intervals affect the response. Of the 4 dogs, 2 usually showed secretion; the other 2 almost invariably did not. The secretion was usually confined to a few areas; in only occasional instances did all of the oral mucous glands react to a single injection. The quantities of mucus which these anesthetized dogs inconstantly produced on the intravenous injection of from 0.25 to 1.0 cc. of a 1/10,000 solution of epinephrine were very minute in comparison with the secretion elicited by a drop of 0.5 N.

HCl upon the tongue of a lightly anesthetized dog, or by the gag reflex in an unanesthetized animal. After the intravenous administration of 4 mg. of atropine, epinephrine injections still called forth a very weak response in 2 of the 4 dogs observed in the majority of experiments. For comparison, direct faradic stimulation of the cervical sympathetic trunk in the neck was performed on all 4 dogs under morphine and ether anesthesia and produced in these animals partial or complete unilateral mucous glandular secretion both before and after the intravenous administration of 4 mg. of atropine.

Conclusion. The responses of the oral and pharyngeal mucous glands to injections of pilocarpine, atropine, and morphine and of the oral glands to epinephrine, resemble those of the salivary glands, an observation which can be explained on the basis of the similar dual innervation of both groups of glands through the cranial autonomic and sympathetic systems.

7373 P

Effect of Simple Duodenal Obstruction upon the Combined Gastric, Pancreatic, Biliary and Duodenal Secretions.*

M. LAURENCE MONTGOMERY AND JOSEPH M. SWINDT. (Introduced by C. D. Leake.)

From the Department of Surgery, University of California Medical School, San Francisco.

Dragstedt and Dragstedt[1] found in anesthetized animals prepared for the simultaneous collection of gastric, pancreatic and duodenal secretions that the intravenous injection of sublethal doses of fluid obtained from an obstructed duodenum or a closed intestinal loop produced "a striking increase in all of the secretions . . . . " Ivy *et al.*[2] later reported that in 7 animals with Pavlov pouches of the stomach the production of a pyloric stenosis decreased at first the secretion of the pouch in response to a test meal. Later 4 of the 7 animals showed a moderate "hypersecretion". Dragstedt, Mont-

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* This work was conducted under grants from the Christine Breon Fund for Medical Research and the Melville Luther Montgomery Fund for Medical Research of the University of California.

[1] Dragstedt, L. R., and Dragstedt, C. A., *J. Am. Med. Assn.*, 1922, **79**, 612.

[2] Ivy, A. C., Droegemueller, E. H., and Meyer, J. L., *Arch. Int. Med.*, 1927, **40**, 434.

gomery, Ellis and Matthews[3] stated that the experiments referred to above[1] "suggest that under conditions similar to those existing in acute dilatation of the stomach, there occurs an absorption of toxic substances from the duodenum which have a pronounced secretogogue action". Recently Sungchin Kim[4] observed an increase in the amount of gastric juice in dogs with a duodenal obstruction.

These experiments differ from the conditions seen in patients with acute intestinal obstruction in so far as the use of anesthesia and the intravenous injection of loop fluid[1] are abnormal and the influence of distention as a possible factor in secretion is absent in the Pavlov pouch experiments. We have, therefore, reexamined the subject under conditions which somewhat more naturally simulate the disorder.

In 7 healthy dogs weighing between 10 and 18 kg. the duodenum was divided below the lower pancreatic duct. A Dragstedt type of intestinal cannula was placed in the duodenum proximal to the division. From this cannula the combined gastric, pancreatic, biliary, and duodenal secretions were allowed to run freely into a clean rubber bag. Collections were made every one to 5 hours. The animals were muzzled or their salivary ducts were ligated in order to reduce or eliminate saliva as a factor. No food was given. Two of the animals were discarded because the ingestion of gauze and other materials caused a premature blocking of the cannula. The other 5 may be divided into 2 groups.

Two dogs belong to the first group. In these the drained secretions were discarded and replacement was made by the subcutaneous or intravenous injection of Ringer's or 5% glucose solution. After a level of secretion was well established duodenal obstruction was produced by dividing the duodenum between the lower pancreatic duct and the drainage cannula. This left an isolated, draining duodenal loop 5½ and 6 cm. long respectively. The obstruction was allowed to continue for 4 and 7 days. Then the animals were sacrificed. The vomitus and the secretion from the isolated duodenal loops were measured and prorated over the period of the obstruction. It was found that the secretion during obstruction as thus measured was somewhat higher than during the control period.

Three animals belong to the second group. In them the secretions were returned during the control period through a cannula placed in the bowel below the division of the duodenum. Duodenal obstruction was produced by capping the cannula and preventing leakage about it, after which replacement was made by the subcutaneous injection of Ringer's or sodium chloride solutions. Following periods of obstruction of 71½ to 93½ hours the animals were either sacrificed or the duodenal cannula was opened and the accumulated fluid allowed to drain for a period of time. This fluid plus the vomitus was then prorated over the period of the obstruction. In all 3 animals the average secretion during the period of obstruction was slightly less than that found during the control period (Table I).

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[3] Dragstedt, L. R., Montgomery, M. L., Ellis, J. C., and Matthews, W. B., *Surg., Gynec. and Obstet.*, 1931, **52**, 1075.

[4] Kim, Sungchin, *Korean Med. J.*, 1932, **2**, 86; *Abstr.: Chem. Abstrs.*, 1933, **27**, 1675.

TABLE I.

Dog No.	Before Obstruction cc. per hr.	 Total hrs.	After Obstruction cc. per hr.	 Total hrs.
39	36.5	59	34	71.5
17	34.4	72	30.7	93
46	45.25	36	43	93.5

In certain other experiments we observed that during periods of free drainage, the secretion is greater when the juices are returned than when they are discarded and replacements are made by salt solution. We feel, therefore, that the second group reflects more exactly the usual condition, and that we must assume therefrom that simple duodenal obstruction does not result in a significant increase of the combined secretion of digestive juices above the fasting level.

7374 C

Psittacosis in the Native Australian Budgerigars.

K. F. MEYER AND B. EDDIE.

From the George Williams Hooper Foundation, University of California, San Francisco, California.

Soon after the discovery of latent psittacosis in the breeding establishments of California the sanitation of the aviaries became a paramount problem. A proposal was made to destroy the flocks which were proven to be infected and to resume breeding in the disinfected pens with a native stock imported from Australia. Such a plan might be feasible only provided the native birds should be free from disease. According to reliable reports no psittacosis has been observed or reported from the Australian continent. It was thus anticipated that the budgerigars would possess a high susceptibility to the virus and would therefore lend themselves for exposure and immunity tests.

Permission was obtained to export 200 shell parrakeets through the port of Sydney. The birds had been trapped during the month of June in the vicinity of Adelaide a few days before they left the country. They had no contact with other birds either on land or at sea. On their arrival in 8 large cages, in July, 1933, the green, partly mature and partly immature budgerigars appeared perfectly healthy and remained so under strict isolation in a special room in a separate building removed from any psittacosis work which was in progress.

One month after arrival one bird died in Cage 4. The autopsy was inconclusive but mice inoculated with the spleen and liver suspensions sickened on the 5th to the 7th day and when sacrificed presented enlarged spleens. Aerobic and anaerobic cultures remained sterile. By passage experiments and filtration tests a virus was obtained which produced the typical lesions in mice and infected rice birds by inoculation and *per os*. The inclusion bodies (L. C. L. bodies) were readily demonstrated. Following this unexpected discovery of the psittacosis virus 10 birds each from Cages 3, 4, 5, 6, 7, and 8 were sacrificed and examined by mouse inoculation tests. In 8 shell parrakeets the virus was present in the spleens and livers and in the nasal mucosa also of one other bird. (Table I.) The highest percentage was found in Cage 4, which housed the majority of immature birds. Only one mature budgerigar (female, Cage 3) presented the anatomical lesions of psittacosis in the form of an enlarged spleen and liver. However, the virus was not found in the tissues. In fact, contrary to the experience gathered on Cali-

TABLE I.
Latent Psittacosis Infections in Australian Shell Parrakeets.

Cage	No. Examined	Anatomically Suspicious	Age	Virus Demonstrated	Size of Spleen
1	2	0	Mature	0	
2	2 (1 died)	0	”	0	
3	10	1 (7x5 mm.)	Early mature	2 (passage)	2.5x2.5x2.5x3
4	14	8	3 ”	6 (nasal mucosa)	2.5x3; 4x3.5; 4.5x5
	1 dead	1	12 immature	1	5x5; 3x3; 4.5x3
5	10	1 (4x4 mm.)	All mature	0	
	1 dead	0	——	1	
6	10	1 (4x4.5 mm.)	Mature	0	
7	10	1 (4x4.5 mm.)	9 ” 1 immature	0	
8	10	0	Mature	0	

fornia parrakeets, it was noted that the size of the spleen is not a definite criterion for a latent infection. Moderately enlarged (2.5 to 5 mm.) spleens classified according to California standards as normal were infectious. Cage 5 furnished non-infected birds but 5 months after the test one parrakeet died and was found to harbor the virus. In order to test the infectiousness of the birds in Cage No. 4 with the highest incidence of latent disease, 4 ricebirds were exposed for 58 days. The birds remained well although 2 of the Australian parrakeets were proven to be carriers. While absolute proof is lacking, it is reasonable to believe that the imported birds should be classed as closed carriers.

The infective agent when first secured was relatively weak. The mice inoculated with the spleens became definitely sick around the 10th to the 14th day. Few died but when sacrificed at the time of the illness or on the 20th day after clinical recovery the virus was invariably demonstrated in the spleen by inoculation and by the presence of L. C. L. bodies in the impression smears from the organs. On the 4th passage the virulence was slightly enhanced and rice birds injected with 1 cc. of a 20% suspension of mouse spleen succumbed on the 6th, 8th, 13th, and 19th days respectively. A total of 11 mouse passages was made within 23 weeks (7 California viruses were simultaneously passaged 10 times in 13 weeks). California raised immature shell parrakeets were readily infected with the Australian virus. Some died in from 6 to 50 days and conveyed the disease to others which were exposed in the same cage.

Incomplete cross-protection tests suggest a close relationship to the California virus. In view of the existence of latent psittacosis it was anticipated that the susceptibility of the Australian parrakeets would be low. Two fatal and 6 carrier infections were produced in 8 Australian budgerigars which had been injected with a ricebird passage virus fatal for mice in a dilution of 10^{-5} on the 11th day. On the 57th day 2 carriers yielded the virus on the nasal mucosa and 3 in the contents of the cloaca. Thirty-nine shell parrakeets from 5 different cages were then injected intramuscularly with ½ cc. of a 20% mouse spleens California parrakeet passage virus which killed mice in dilutions of 10^{-7} on the 12th day (Table II). Of 10 deaths 6 were conclusively attributed to the psittacosis infection; 2 birds died from relapses on the 74th and 98th days although the anatomical lesions were suggestive of an acute process. Only 8 of the 29 surviving parrakeets were proven carriers. The recovery rate during the period (37 to 146 days) was 73%. This figure corresponds closely to that of 77% established in a similar

TABLE II.
Susceptibility of Australian Parrakeets to California Virus.

Cage No.	No. Birds	Deaths	Carriers	Non-infected
1	8	2 (9th and 25th days)	6 (57th day)	0
	Exposure 4	1 (106th day)	0	3 (128th day)
	” 2	1 (25th day)	—	1 (25th ”)
3	6	2 (21st and 74th days)		
		1 (negative 40th day)	—	3 (83rd ”)
4	6	1 (36th day)	—	3 (83rd ”)
		2 (negative 36th day)	—	
5	10	1 (48th day relapse)	1 (49th ”)	2 (49th ”)
		7x7.5 mm.	1 (83rd ”)	2 (83rd ”)
				3 (146th ”)
6	7	1 (98th day)	1 (79th ”)	2 (79th ”)
		8x11 mm.		3 (83rd ”)
7	10	1 (14th day)	3 (37th ”)	2 (37th ”)
		1 (negative 104th day)	1 (83rd ”)	1 (83rd ”)
			1 (146th ”)	
	53	10 positive 4 negative (26%)	14 (27%)	24 (47%)

experiment on mature California budgerigars from an infected aviary. In exposure experiments in which 6 parrakeets were brought in contact with sick and dying ricebirds only 2 contracted psittacosis. Twenty-four budgerigars from Cages 1 and 2 mingled for 174 days with a flock of California parrakeets in which the incidence of latent infection was estimated at 50%. None of the exposed birds died and only one revealed at autopsy an enlarged spleen without virus. The nature of this resistance is unknown and since it is not associated with neutralizing antibodies it is difficult to determine whether it is the result of a sub-clinical infection or of maturation immunity. Observations on California shell parrakeets from infected and non-infected breeding establishments indicate a lower recovery rate in the birds from psittacosis-free stock. The differences are sufficiently striking to suggest that a higher recovery rate follows previous contact with the virus.

The demonstration of latent infection together with a high resistance and recovery rate strongly supports the conclusion that psittacosis is an endemic disease of the native Australian parrakeet. The birds examined were closed carriers of a weak virus. California experiences indicate that such infections fail to give rise to human psittacosis. However, it must always be remembered that missed human infections particularly in the owners of aviaries and pet shops are by no means uncommon. In the light of the newer knowledge these groups deserve further scrutiny on the Australian Continent.

7375 P

Observations on Fowl Paralysis (Neurolymphomatosis)*

J. FURTH.

From the Department of Pathology, Cornell University Medical College, New York.

There are many reports on transmission experiments with fowl paralysis. Some workers have found a greater incidence of paralysis in chickens inoculated with tissues from paralyzed chickens than in uninoculated controls; *e. g.*, Pappenheimer, Dunn and Seidlin[1] found paralysis in 25% of the inoculated chickens and 7% of the uninoculated controls. Similar but somewhat more successful were the transmission experiments of Dalling and Warrack.[2] Biely, Palmer and Asmundson[3] found that the incidence of paralysis depends on inherited susceptibility, and is not conspicuously influenced by inoculation with tissues from paralyzed chickens. There is no report of a readily transmissible strain of fowl paralysis and the relation of fowl paralysis to leukosis is not clearly defined in the literature.

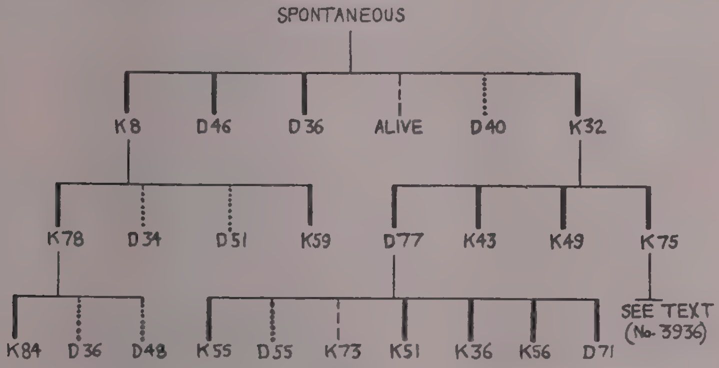

CHART 1.

| = neurolymphomatosis. ¦ = inoculation unsuccessful.
⁞ = died within 2 months after inoculation and showed no gross alterations characteristic of neurolymphomatosis.
D = died. K = killed. The numbers show in days the length of life after injection.

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*This investigation has been supported by a Fund for the Study of Leukemia. Mr. C. Breedis assisted in the work.

[1] Pappenheimer, A. M., Dunn, L. C., and Seidlin, S. M., *J. Exp. Med.*, 1929, **49**, 87.

[2] Dalling, T., and Warrack, G. H., *Atti del V Congresso Mondiale di Pollicoltura,* 1933, No. 90.

[3] Biely, J., Palmer, E., and Asmundson, V. S., *Canad. J. Res.*, 1932, **6**, 374.

Two transmissible strains of fowl paralysis have been isolated. Of one strain (Strain 5) 15 successful passages were made, of the other (Strain 6) 10 passages were made. Chart 1 shows only the results of inoculations of the first 5 passages of Strain 5.

The inoculations were made in most instances by intravenous injections of blood from paralyzed chickens, into healthy young chickens. The transmitting agent circulates in the blood of paralyzed chickens throughout the course of illness as shown by the following experiment:

No. 3946 was inoculated and showed beginning paralysis of legs and wings 42 days later. At this time three chickens were injected with 0.5 to 5 cc. of its blood. Two of these chickens developed paralysis and the third remained healthy. Eighteen days later the signs of paralysis were more conspicuous and another two chickens were injected with 0.5 to 5 cc. of blood of chicken No. 3946. Both of these chickens developed paralysis. Thirteen days later both extremities and one wing of No. 3946 were completely paralyzed, and the chicken was killed *in extremis;* two more chickens were injected with 0.5 cc. of its blood and both died with neurolymphomatosis.

Thus in contrast to common virus diseases and as with Rous sarcoma and chicken leukosis, antibodies do not seem to interfere with the circulation of the agent in the blood of the affected chickens. Nevertheless chickens that failed to develop the disease after one injection resisted repeated intravenous reinoculations.

After intraneural inoculation lymphomatous infiltrations cause great thickening of the injected nerves. After injection into the sciatic nerve the infiltrations extend distally from the site of injections. Since after intraneural injection the lesions in uninjected nerves are similar to those found after intravenous injection and the infiltrations in the nerve proximally to the site of injection are inconspicuous, it is probable that the generalized disease is produced by hematogenous and not by neural dissemination of the agent.

Neurolymphomatosis, unlike all known transmissible strains of leukosis of chickens, does not affect the bone marrow, rarely affects the liver and the spleen, and rarely causes conspicuous alteration in the number of the circulating leukocytes. Occasionally, the number of lymphocytes in the blood is increased.[4] The highest leukocyte count observed was 140,000 and 92% of these cells were lymphocytes, mostly of small size. This leukocyte count was found in the chicken from which Strain 5 took its origin. The cells that infiltrate the nerves are mainly lymphocytes of small and medium size, and the infiltration has the characteristics of a neoplasm. Moreover, neurolymphomatosis is often associated with lymphomatous infiltrations of viscera such as ovary, lung and heart, but the liver is seldom infiltrated. These characteristics differentiate neurolymphomatosis from the most common type of lymphomatosis of chickens which is associated with great enlargement of the liver caused by infiltration with large round cells like lymphocytes; this disease may be called hepatolymphomatosis. Hepatolymphomatosis is not associated with paralysis. Lymphomatosis caused by our Strain 2[5] is characterized by the presence of numerous large basophile lymphocytes in the blood and the same large cells often infiltrate nerves and ganglia.

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[4] *Cf.* Biely, J., Palmer, V. E., and Lloyd, E. A., *Atti del V. Congresso Mondiale di Pollicoltura,* 1933, No. 89.

There are several agents that cause leukosis and their characteristics remain constant throughout numerous animal passages. To the 2 types of agents of leukosis described in previous communications[5] a third type of agent may be added, namely that which produces neurolymphomatosis. The agent causing hepatolymphomatosis has not yet been discovered.

7376 P

Transmission of Myeloid Leukemia in Mice.*

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From the Department of Pathology, Cornell University Medical College, New York.

Myeloid leukemia, the most common type of leukemia in man, is rare in animals. A survey of this disease leaves doubt whether myeloid leukemia has ever been observed in domesticated animals (Jármai[1]). It has been described in mice by Simonds,[2] although neither the bone marrow nor blood smears were available to him.

Myeloid leukemia was rarely found among our mice until a large number of them had been exposed to a single dose of approximately 400 r-units of X-rays or to smaller doses repeated at approximately

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[5] *J. Exp. Med.*, 1933, **58**, 253.

* This investigation has been supported by a Fund for the Study of Leukemia.

[1] Jármai, K., *Ergebn. allg. Path. u. path. Anat.*, 1934, **28**, 227.

[2] Simonds, J. P., *J. Canc. Res.*, 1925, **9**, 329.

TABLE I.

	No. of Mice	
	Irradiated	Control
Mice over 8 months of age examined post mortem	385	481
Died with myeloid leukemia	21	2
Died with mediastinal lymphosarcoma	62	9
Died with either lymphoid or myeloid leukemia	118*	37*
Died with malignant tumors other than lymphosarcoma	23	40

* These figures include those of the second and third groups.

monthly intervals. Table I contains a preliminary survey of this experiment and shows the frequent occurrence of myeloid leukemia and mediastinal lymphosarcoma among the irradiated mice as compared to the unirradiated, closely related control mice.

Increase of spontaneous lymphomatosis in mice by exposure to X-rays was observed by Krebs, Rask-Nielsen and Wagner.[3] Aubertin[4] called attention to the frequent occurrence of myeloid leukemia in X-ray workers.

The mouse from which the first strain of myeloid leukemia of mice took its origin was an unirradiated male mouse (No. Ar117). At the age of 16 months, the spleen of this mouse was greatly enlarged while the lymph nodes were of normal size or only slightly enlarged. The leukocyte count was 105,000. The most significant feature of the differential count was the presence of 9.5% of immature myeloid cells with coarse basophile granules in the blood. Neutrophile polynuclear leukocytes were 31%, neutrophile myelocytes and metamyelocytes 20%, eosinophile granulocytes 0.5%, cells like monocytes 17%, cells like monocytes with basophile cytoplasm 11.5%, lymphocytes 10.5%. At post mortem examination the liver, bone marrow, and spleen showed extensive infiltrations by myeloid cells with basophile granules and the same cells filled the sinuses of many lymph nodes. Many of these myeloid cells were in the process of mitotic division.

TABLE II.

	Injection	No. in Group	No. successful Inject.	No. unsuccessful Inject.
Unrelated to mouse with spontaneous disease; irradiated*	Intravenous	4	3	1
Unrelated; irradiated	"Intrasplenic"	4	4	0
Related; not irradiated	Intravenous	8	1	7
" " "	"Intrasplenic"	4	0	4

*With 400 r-units of X-rays 24 hours before inoculation.

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[3] Krebs, C., Rask-Nielsen and Wagner, A., *Acta radiologica*, Suppl. to Vol. 10, 1930.

[4] Aubertin, C., *Bull. off. Soc. Franc. d'electro. et de radiol.*, 1931, **40**, 218.

The spleen of this mouse was cut up in the presence of Locke's solution, filtered through a small piece of cotton and injected into 20 mice as shown in Table II.

After shaving the skin of mice over the splenic area and moistening the skin with alcohol, the spleen becomes visible. With a 27 gauge needle inoculation was attempted into the spleen through the intact abdominal wall. In all 4 irradiated mice gray tumors developed about the spleen and in the subcutaneous tissue at the site of injection, and these tumors were composed of immature myeloid cells with coarse basophile granules.

After intravenous inoculation the spleens of the injected irradiated mice were found to be much enlarged within from 13 to 18 days after the injection. Post mortem examination showed that this organ was almost completely replaced by immature myeloid cells with coarse basophile granules, and diffuse infiltration with these cells caused great enlargement of the liver. The lymph nodes were only slightly enlarged.

Subpassages were readily made by intravenous or subcutaneous inoculation of emulsions of cells obtained from the infiltrated spleen. Of the 46 irradiated mice injected intravenously, 42 developed myeloid leukemia in the course of the first 4 subpassages. After subcutaneous inoculation tumors developed at the site of injection. After intravenous inoculation the systemic disease that was produced was characterized by extensive infiltrations of several organs, chiefly, spleen, liver and bone marrow, with cells similar to those that infiltrated the organs of the mouse with the spontaneous disease. Many of these cells were in the process of mitotic division. They appeared in the circulating blood of all mice inoculated intravenously and leukemia was associated with very severe anemia. *E. g.*

No. R 3143 was inoculated 24 hours after irradiation, and 18 days after injection the leukocyte count was 34,000, of which 94.5% were the immature, presumably malignant myeloid cells with coarse basophile granules. The erythrocyte count was reduced to 494,000, the lowest erythrocyte count we have observed in mice.

These experiments indicate (a) that the myeloid leukemia studied by us is transmissible, (b) the leukemic myeloid cells, like the leukemic lymphocytes,[5] have the characteristics of neoplastic cells and multiply but do not mature into normal leukocytes, (c) they produce systemic disease after intravenous inoculation and form tumors (myelomata) after subcutaneous injection.

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[5] *Cf.* Furth, J., Seibold, H. R., Rathbone, R. R., *Am. J. Canc.*, 1933, **19**, 521.

7377 C

Action of Sodium Salicylate on Fermentation of Salicin and Glucose by Streptococci.

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The action of sodium salicylate on the biological functions of streptococci is of particular interest because of the obviously beneficial effects obtained through the administration of sodium salicylate in rheumatic fever.[1, 2] The etiology of this disease is still considered to be doubtful, yet streptococci seem to be involved, at least in producing complications. Experiments on the direct streptococcidal action of salicylates indicated that this action is not sufficiently strong to explain any curative effect.[3] Curative effect was not found in animal experiments.[4] The opinion that salicylates act in clinical cases mainly by virtue of their antipyretic and analgesic properties has thus come to be almost generally accepted.[5, 6] However, this is in contradiction to the fact that other antipyretic and analgesic drugs cannot as a rule replace salicylates in rheumatic fever.

Studies on the mechanism of the action of different chemotherapeutic agents (neoarsphenamine, Bayer 205) have indicated that in low therapeutic concentrations these do not kill the parasites but merely decrease their virulence.[7] While the activity of these agents can be easily demonstrated in animal experiments, salicylates do not seem to be active in the experimental infections so far studied. This may be due to the fact that there is no experimental infection of streptococci which is strictly comparable with rheumatic fever. The writers have studied skin lesions produced by the intradermal injection of streptococci (green producing and hemolytic) and by the

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[1] Meyer, H., Gottlieb, R., and Henderson, V. E., *Experimental Pharmacology*, Lippincott, 1926, 562.

[2] Caussade, Q., and Tardieu, A., *Revue médicale*, 1931, **48**, 287.

[3] Birkhaug, K. E., *J. Infect. Dis.*, 1931, **48**, 212.

[4] Miller, J. L., *J. Am. Med. Assn.*, 1914, **63**, 1107.

[5] Swift, H. F., and Boots, R. H., *J. Exp. Med.*, 1923, **37**, 553.

[6] Davis, D. J., *Arch. Int. Med.*, 1915, **15**, 555.

[7] Reiner, L., and Koveskuti, J., *Deut. Med. Wochenschr.*, 1927, **53**, 1988. Reiner, L., and Leonard, C. S., *Arch. intern. pharmacodynamie*, 1932, **43**, 10, 49.

same organisms treated *in vitro* with sodium salicylate. There was no appreciable difference between the lesions produced by salicylate-treated streptococci and controls. Neither was any effect observed on similar skin lesions in 2 rabbits which received 10 daily injections of one gram each (5 cc. of 20% solution) of sodium salicylate subcutaneously. Seven injections were given prior to and 3 following the intradermal injections of streptococci.

A definite damaging action of sodium salicylate was found, however, when the glucose and salicin fermentation of streptococci was studied in the presence of sodium salicylate. The extent of fermentation was estimated by the total acid produced and by the change of pH of the medium.* Details and results of a typical experiment are given below.

Streptococcus, AB, 13,† was grown for 18 hours in beef heart broth (pH 7.4); centrifuged and the sedimented organisms suspended in one-twentieth of the original volume of peptone solution. Two-tenths cubic centimeter of the heavy culture suspension was added to tubes containing salicin or glucose, both with and without sodium salicylate in a medium of beef extract broth. All tubes were brought to equal volumes (9.0 cc.). After incubation for specified times, a portion of each solution was removed for testing. pH determinations were done by colorimetric method. Non-volatile acid production was determined by titration with N/10 sodium hydroxide with phenolphthalein as indicator, after preliminary addition of a definite amount of hydrochloric acid and boiling to remove CO_2.

The experiment tabulated shows that 0.5% sodium salicylate strongly inhibited the fermentation activity of streptococcus AB, 13, for salicin and glucose. Distinct inhibition was also found with 0.2% salicylate. The pH change and the acid produced in 24 hours was always less in the presence of sodium salicylate than the pH change and acid produced in 4 hours without salicylate. The inhibition of activity by sodium salicylate for salicin was greater than for glucose.

The growth of bacteria after 6 hours incubation in these various

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* The inhibiting action of salicylic acid on the fermentation of sugar by yeast was demonstrated by Kolbe.[8] For the action of salicylates on other ferments, see Hanzlik.[9] The activity of yeast lactic dehydrogenase is not inhibited by sodium salicylate (our own experiment).

[8] Kolbe, H., *Chem. Z.*, 1874, 617, 632.

[9] Hanzlik, P. J., *Actions and Uses of the Salicylates and Cincophen in Medicine*, The Williams Wilkins Co., 1927.

† This culture was obtained through the courtesy of Dr. Nicholls and Dr. Stainsby, New York Hospital, Cornell Medical College, New York.

TABLE I.

Fermentation Activity of Hemolytic Streptococcus for Salicin and Glucose with and without the Addition of Sodium Salicylate.

		Observations after Incubation for Various Periods of Time						
		4 hrs.	6 hrs.				24 hrs.	
Carbo-hydrate .5%	% Sodium Salicylate	pH	pH	Density	colonies per cc. in millions	Approximate number of cocci per chain or clump	pH	Total Acidity for 10 cc.
Salicin	—	5.3	5.0	5	4,400	12-20	4.9	2.36*
,,	0.5	6.4	6.4	3½	3,400	8-12	6.3	1.20
,,	0.2	6.3	6.3	3½			5.4	1.64
Glucose	—	5.0	4.9	5	3,140	16-20	4.8	2.72
,,	0.5	5.6	5.5	3½	2,960	8-12	5.4	1.82
,,	0.2	5.4	5.4	4			5.3	2.12
†—	—	6.7	6.7	3½	3,040	8-12	6.4	0.68
—	0.5	6.7	6.7	3	2,780		6.4	0.84

* In terms of tenths milli-equivalents of acid produced per 10 cc. of culture.

† Before incubation, Density = Barium Sulfate Standard No. 3.
Colonies per cc. = 4,000 million.
Approximate number of cocci per chain, 8-12.

media showed interesting differences when measured by the density, smear examination and colony count in blood agar plates. The density in the tubes in which the organisms were grown in salicin or glucose alone had increased from the original value of No. 3 (Barium Sulfate Standard) to a value of 5, while in the tubes which contained sodium salicylate with the sugars, the density had increased only to final values of 3½ to 4. Examination of smears made at this time showed that in the tubes containing carbohydrate, but no salicylate, the number of cocci per chain had increased from the original average number of 8-12 to an average of 12-20 and there was a tendency to form clumps. The number of chains or clumps per field, however, appeared to be unchanged. In the tubes containing salicylate, 0.5%, with the carbohydrates, the number of cocci per chain and the number of chains per field were approximately the same as in the original culture dilution. On the other hand, the number of colonies per cubic centimeter, as evidenced by the growth in blood agar pour plates, incubated for 48 hours was similar in the tubes with or without sodium salicylate. This might be taken as an indication of the fact that the colony count alone is inadequate as a method for studying the growth of streptococci. It is of interest to note that streptococci with short chains sometimes have been considered to be less virulent.

The fermentation of salicin and that of glucose by streptococci was found to be inhibited by sodium salicylate. The chains were shorter in the presence of salicylate than in the controls without salicylate. The density was also less in the former case than in the latter, largely due to the fact that there were fewer individual cocci, although the number of chains or clumps were approximately the same in both cases as shown by the plate count. It is conceivable that this inhibition of a very important biological function of the streptococci is associated with a decrease of their vitality and their virulence.

The writers wish to express their thanks to Dr. W. H. Park for his interest and advice.

7378 P

Cephalin Content of Prepared Fibrinogen and Prothrombin Solutions.

JOHN H. FERGUSON. (Introduced by H. E. Himwich.)

From the Laboratory of Physiology, Yale University School of Medicine.

Mills[1] prepared fibrinogen and prothrombin solutions from platelet-free plasma; the fibrinogen remained unclotted by both calcium and cephalin for 24 hours at 40°C.; the prothrombin needed cephalin as well as calcium for its activation. Few other investigators have secured preparations which could fulfil these rigorous tests, and Howell,[2] in particular, has held to the idea that calcium alone is sufficient to activate prothrombin, the more especially since he and Cekada[3] were able to obtain active thrombins that were lipoid-free. In view of Mills' demonstration that (a) the prothrombin prepared by Howell's acetone method contained cephalin which could be extracted with cold benzene, leaving a preparation which needed cephalin as well as calcium for its activation, and that (b) an appreciable amount of cephalin-like lipoid could be extracted by boiling ether from platelet-free goose and dog plasma, we wondered whether Howell's pure thrombins had been tested on fibrinogen solutions free from lipoids. The following experiment emphasizes the need for satisfying this inquiry.

A dog, *not fasted*, was anesthetized with sodium amytal. Blood was collected, via a paraffined cannula in the carotid artery, into cooled paraffined centrifuge tubes containing sodium citrate (to a final concentration of 0.5%). After immediate low speed centrifugalization to remove red and white cells, the platelets were separated by prolonged high speed centrifugation. The plasma was salted with 15% (solid) NaCl, followed by 18% $(NH_4)_2SO_4$, the respective fibrinogen and prothrombin fractions being purified by washing and reprecipitation, and tested for their coagulation reactions. The final solution of prothrombin was found to be activated by calcium alone, and formed a firm clot with fibrinogen solution in 4-5 minutes. After extraction for a few minutes with cold benzene, calcium was unable to activate the coagulant. The recalcified benzene-extracted prothrombin, however, still clotted unextracted fibrinogen solution, although somewhat more slowly (10 min.). If the benzene extract (from the fibrinogen solution) was layered on top of a mixture of the recalcified 'lipoid-free' reagents, clotting started in about 10 minutes at the benzene surface and slowly spread down the tube through the watery layer. A small quantity of benzene extract, evaporated to dryness, gave an almost invisible residue which, when dissolved in distilled water, readily activated the recalcified extracted prothrombin. Pure cephalin (from brain) acted in the same manner, but lecithin *(de ovo)* was without effect.

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[1] Mills, C. A., *Chinese J. Physiol.*, 1927 (several papers).

[2] Howell, W. H., *Am. J. Physiol.*, 1910, **26**, 453.

[3] Cekada, E. B., *Am. J. Physiol.*, 1926, **78**, 512.

We conclude that tests with cephalin-free fibrinogen solutions must be specifically included before evaluating the coagulant properties of highly purified thrombin preparations. Wooldridge[4] was fully aware that the protein fractions obtained by salting contained "lecithin" (the current term for phosphatides). He tried in vain to obtain lipoid-free fibrinogen, and in an approximate analysis determined 3.2% of lecithin (dry weights). Theorell[5] also found 0.623 gm. of ether soluble P per gram of protein in horse plasma fibrinogen obtained by salting with 27% of ammonium sulphate.

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[4] Wooldridge, L. C., Collected papers on "The chemistry of the blood," 1893.

[5] Theorell, A. H. T., *Biochem. Z.*, 1926, **175**, 297.

7379 P

Electrocardiographic Evidence of Cardiac Involvement in Acute Disease.

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Daily electrocardiograms were taken on patients suffering from acute disease who received no digitalis or quinidine. (Table I.) No such systematic electrocardiographic study has been made except in rheumatic fever,[1] pneumonia,[2] and acute rheumatoid (infectious) arthritis.[3] The recorded abnormalities were produced during the illness. Transient changes in lead III only were not included in the table.

For control data, 50 cases of acute rheumatoid (infectious) arthritis, 6 cases of ulcerative colitis and 9 cases of peptic ulcer were chosen. In the former 2 groups the patients were acutely ill with fever. In all the control patients no abnormalities in T-, QRS-waves, or in RS-T transitions, no auricular flutter or fibrillation, no heart-block were observed. Hence, one may conclude that an acute illness with fever and tachycardia does not produce significant arrhythmias, increased P-R intervals, T-wave inversions or marked RS-T changes unless the myocardium is involved.

Most marked heart-block and the most frequent arrhythmias were observed in acute rheumatic fever but a larger number of patients with this disease was studied than in any other. In acute disseminated lupus erythematosis, in periarteritis nodosa, in Malta fever, in capillary toxicosis, bronchopneumonia, typhoid and acute rheumatic fever, T-wave changes were most frequent. It is suggested that some of these diseases may have similar pathological alterations in the myocardium of vascular nature and that they may produce permanent heart damage.

A very fast pulse occurred in many cases with severe myocardial involvement. In 7 cases of pulmonary tuberculosis, for example, with rates above 120 beats per minute, 6 disclosed abnormal electrocardiograms. In this disease the occurrence of a heart rate of over 120 beats per minute should suggest myocardial involvement. However, a slow pulse did not indicate a good myocardium, for

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[1] Cohn, A. E., and Swift, H. F., *J. Exp. Med.*, 1924, **39**, 1.

[2] Master, A. M., Romanoff, A., and Jaffe, H., *Am. Heart J.*, 1931, **6**, 696.

[3] Master, A. M., and Jaffe, Harry, *J. A. M. A.*, 1932, **98**, 881.

TABLE I.

	No. Patients	Aver. No. Records	Tachycardia	Bradycardia	Arrhythmias*	P-R over 0.20 sec.	Maximum P-R	T-wave Inversions	RS-T Changes
Acute rheumatic fever	63	30	68%	21%	37%	53%	.56	28%	85%
Typhoid	15	25	87	27	13	33	.24	27	33
Acute Lupus Erythematosis	6	16	100		17	33	.24	50	50
Typhus	14	9	64	28		35	.28	7	14
Lobar pneumonia	45	15	69	51	10	11	.24	15	93
Bronchopneumonia	8	15	50	13			.20	37	13
Pulmonary Tuberculosis	27	11	42	7	3	11	.24	15	11
Gonorrheal Arthritis	16	11	31	6		19	.26	19	13
Capillary Toxicosis	3	14	33					63	33
Periarteritis nodosa	4	7	75					100	
Malta Fever	2	27	100					100	
Trichinosis	4	8	50			25	.22	33	
Erythema nodosum	14	13	50	21	7	14	.23	14	
Malaria	7	7	29	43	14	14	.34		29
Erythema multiforme	5	9	40			20	.22		
Urticaria	6	9		16					
Purpura hemorrhagica	6	9	16	16		16	.21		
Controls									
Ulcerative Colitis	6	9	50	16	16				
Peptic Ulcer	9	9							
Rheumatoid Arthritis	50	30	34	20	2	10	.22		

* Premature beats, sino-auricular block, nodal rhythms, auricular flutter, or fibrillation, heart-block with dropped beats.

bradycardia and heart-block were observed in patients with marked electrocardiographic abnormalities.

The increased A-V conduction time in typhus fever (Brill's disease), occurred usually at the time of the crisis. A similar observation was found in lobar pneumonia.[2]

Unlike the observations in acute rheumatoid (infectious) arthritis one not uncommonly found significant electrocardiographic changes in patients with gonorrheal arthritis.

Cases of erythema nodosum and erythema multiforme revealed very little evidence of myocardial damage and hence their relation to acute rheumatic fever is probably remote.

In none of the diseases studied was a definite progressive and continuous change observed from an RS-T abnormality to an inverted T-wave as is seen following an acute coronary artery occlusion. This may serve as a means of differential diagnosis between acute coronary artery occlusion and other conditions which may produce severe chest, sternal or precordial pain such as pneumonia or pulmonary artery occlusion.

7380 P

Induction of the Olfactory Placode by the Forebrain in Rana pipiens.

EDGAR ZWILLING. (Introduced by L. G. Barth.)

From the Zoology Department, Columbia University.

Bell,[1] using embryos of *Rana esculenta*, attempted to show the relation of the brain to the development of the olfactory placode. He transplanted "that part of the brain which forms the olfactory lobe" to other regions of the body without obtaining any induction of an olfactory placode. His conclusion was that the olfactory placode developed independently of the brain. He did not, however, preclude the possibility of an early stimulus for olfactory placode formation being imparted by the brain to the ectoderm above it.

Recently we have been able to show that the part of the forebrain median and slightly anterior to the optic vesicle of the intermediate neurula stage of *Rana pipiens* has the power to induce an olfactory placode in the ectoderm of the flank (Fig. 1).

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[1] Bell, E. T., *Arch. f. Ent. Mech.*, 1907, **23**.

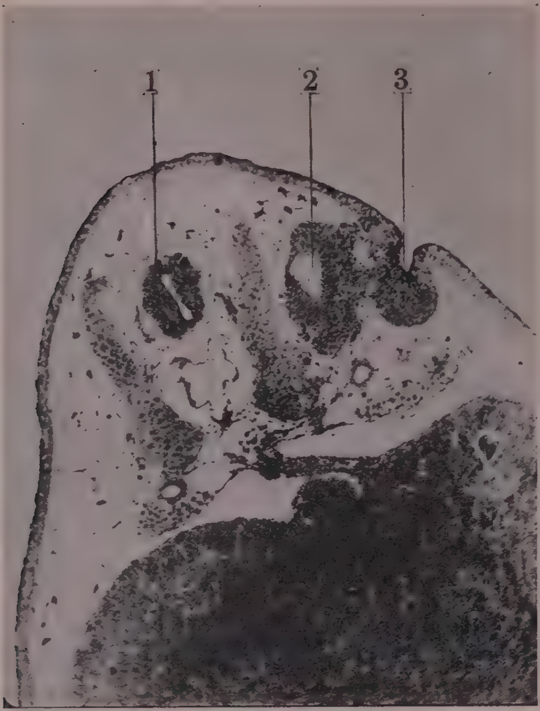

FIG. 1.

Induction of an olfactory placode. 1—spinal cord of host; 2—transplanted fore-brain; 3—induced olfactory placode.

After one accidental induction (from a transplant of an optic vesicle which evidently included some of the rest of the fore-brain) 3 series of operations were performed in an attempt to localize the olfactory placode inducing part of the brain. The following stages were used for the operation:

Early neurula—equivalent to stage 17 on Harrison's scale for *Ambystoma punctatum.*

Intermediate neurula—equivalent to stage 20 on Harrison's scale for *Ambystoma punctatum.*

Late neurula—equivalent to stage 22 on Harrison's scale for *Ambystoma punctatum.*

Early tail-bud—in which the olfactory placode is visible as a pigmented thickening of the ectoderm.

In series 1 and 2 pieces of the fore-brain anterior to the optic vesicle were transplanted to the flank in stages 17, 20 and 22. No definite induction was obtained, although defects appeared in the olfactory placode on the operated side. In series 3 large pieces of the fore-brain of stage 20 median to and almost on a level with the optic vesicle were used. Of the 8 embryos operated on 5 had definite olfactory invaginations appearing in the flank region. Two of the 5 showed well developed olfactory canals connecting with the invaginations. In all cases the olfactory placode on the operated side was missing or rudimentary.

In all of the cases the head ectoderm on one side was lifted up, the required portion of the brain (including the proximal end of the optic vesicle in most cases) was removed, and the head ectoderm was replaced. The piece of brain tissue was transplanted underneath the ectoderm of the flank region of the same embryo. The embryos were sectioned after 3 or 4 days of development.

Some evidence has been obtained which indicates that the olfactory placode is determined in the head ectoderm some time between the late neurula and early tail-bud stages. In series 4 the brain tissue was removed in stages 20, 22 and early tail-bud and the head ectoderm was replaced. No olfactory placode developed except in the case of the early tail-buds. In this stage the placode is already visible and so the removal of the brain does not interfere with its continued differentiation.

Removal of the presumptive olfactory placode in stages 17, 20 and 22 resulted in the formation of an olfactory placode from the ectoderm which healed over the wound. Even after the placode has formed in the early tail-bud stage a second one will form after extirpation. This may be due to a re-induction of an olfactory placode by the brain and not a regeneration as claimed by Bell and by Burr.[2]

7381 P

Measurement of Circulation Time from Antecubital Veins to Pulmonary Capillaries.

WILLIAM M. HITZIG.* (Introduced by B. S. Oppenheimer.)

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Several methods are available for measuring the circulation time from the antecubital veins through the pulmonary bed to the capillaries of the systemic circuit. One method is the decholin method proposed by Winternitz, Deutsch, and Bruell,[1] and recently studied by Tarr, Oppenheimer and Sager.[2] Another is described by Fishberg, Hitzig, and King,[3] in which saccharin injected into an antecubital vein is detected in the tongue. No method has been devised clinically applicable for measuring circulation time from the peripheral veins to the capillaries of the lung. This may be obtained by introducing ether into an antecubital vein and determining the time that elapses before the patient perceives its odor. Ether is peculiarly adapted to this purpose because: 1. Its volatility at blood temperature is so great that when a small quantity is injected, a sufficient amount volatilizes in the pulmonary capillaries during the first circuit of the blood to be invariably perceptible by smell. 2. The small volume needed permits rapid intravenous introduction, the advantage of this being that it will flow in a small blood volume, resulting in a sharp definition of both the time of injection and the time of arrival in the pulmonary capillaries. 3. It is harmless in the quantities used. In over 200 injections, no untoward constitutional reactions were encountered. The subject is usually aware of a "creeping" feeling along the course of the vein. About 25% of the patients complain of transient pain in this location. In 28 cases in which the veins were carefully followed, thrombosis developed on 3 occasions. Paravenous infiltration causes no necrosis. In many instances, the measurement was carried out 2 and even 3 times within a few minutes without causing unpleasant reactions. 4. Because of its volatility, the measurement can be repeated as soon as desired.

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[2] Burr, H. S., *J. Exp. Zool.*, 1916, **20**, 27.

*Eugene Meyer, Jr., Fellow in Pathology.

The determination is performed in the following manner: The individual reclines comfortably with his arm at a level corresponding to the right auricle. He is familiarized at first with the odor of ether. He is then instructed to breathe normally, and to announce when he smells the ether. A mixture of 5 minims of ether and 3 minims of normal saline is rapidly injected into a large antecubital vein, only a fraction of a second being required. The end-point is sharp and unmistakable. Moreover, an observer, in a position close to the subject, can perceive the ether odor almost as rapidly as the patient. This objective confirmation, valuable as it is of itself, becomes of especial importance when studying unconscious patients. To obtain results which will check closely on repetition, the subject must be told to relax and not to hold his breath during the procedure, for this retards the venous return to the heart. Similarly, hyperventilation will hasten the venous return and accelerate the circulation time.

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[1] Winternitz, M., Deutsch, J., and Bruell, Z., *Med. Klin.*, 1931, **27**, 986.

[2] Tarr, L., Oppenheimer, B. S., and Sager, R. V., *Am. Heart J.*, 1933, **8**, 766.

[3] Fishberg, A. M., Hitzig, W. M., and King, F. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 651.

The measurement was performed on 152 individuals, most of whom were hospital patients suffering from various conditions. The end-point was definite in practically every case, an occasional reading only requiring repetition. In 100 "normal" subjects, without evidence of circulatory disturbance, the circulation times showed the following distribution:

Circulation Time (sec.)	Number of Individuals
3½-3¾	7
4 -4¾	32
5 -5¾	27
6 -6¾	23
7 -7½	8
7¾-8	3

Repeated injections checked closely.

In view of the rapid rate of diffusion of a light gas in a gaseous mixture, it may be assumed that the time required for the ether to rise from the pulmonary alveoli to the nasopharynx is negligible in comparison to the actual circulation time.

The "ether time" is thus a measure of the circulation time from the antecubital vein to the capillaries of the lung. It is interesting to compare this circulation time with the "saccharin time", which is a measure of the speed of the circulation from the antecubital vein through the lungs to the capillaries of the tongue. The percentage relationship of "ether time" to "saccharin time" in 27 "normal" individuals showed the following variations:

Ether Time Expressed as % Saccharin Time	Number of Individuals
33-39	4
40-49	15
50-59	6
60-66	2

In a number of the cases studied, thrombosis developed following the consecutive injection of ether and saccharin into the same vein at the same sitting. Consequently, pending further study of the mechanism of such thrombosis, this procedure is not advocated.

The study has shown that in certain varieties of heart failure, the ether circulation time is considerably prolonged. The comparative study of the "ether" and "saccharin time", in various types of heart failure, is of value in localizing the portion of the circulation in which blood flow is retarded. Thus there are instances of left heart failure, in which the "ether time" is within normal limits while the "saccharin time" is much prolonged. This shows that in these cases the retardation of the blood flow is downstream to the arterial capillaries of the lung.

7382 C

Intermediate Products of the Propionic Acid Fermentation.

H. G. WOOD AND C. H. WERKMAN.

Department of Bacteriology, Iowa State College, Ames, Iowa.

The dissimilations brought about by the propionic acid bacteria have received relatively little study. Phosphorylation appears to be a normal process with the intermediate formation of a hexose-monophosphate.[1] Methylglyoxal has been reported by Pett and Wynne[2] and Wood and Werkman have recently identified pyruvic acid[3] and a non-reducing carbohydrate[4] as intermediate products in the fermentation of glucose by *Propionibacterium arabinosum.* Virtanen[5] proposed the occurrence of lactic acid but was unable to detect its presence. Foote, Fred and Peterson[6] have reported the occurrence of lactic acid in the propionic acid fermentation. The intermediate mechanism leading to propionic acid is unknown. The fermentation of glycerol offers an opportunity of studying this phase. In this remarkable fermentation, glycerol is converted under anaerobic conditions practically quantitatively into propionic acid. No gas is produced (van Niel[10]).

To detect intermediate compounds the following medium was fermented: Yeast extract (Difco) 0.5%, glycerol 2.0%, and calcium carbonate 2.0%. Calcium sulfite (1.0%) was included in the medium as a fixing reagent. *P. arabinosum* was the species employed. The fermentation took place under anaerobic conditions at 30°C. After 5 or 6 days' incubation, the fermentations were tested for aldehydes and ketones by addition of 2, 4-dinitrophenylhydrazine. An abundant precipitation was obtained. Distillation of the medium according to Peterson and Fred[7] gave a quantitative recovery of the unknown carbonyl compound. Derivatives were prepared from concentrated distillates and purified by crystallization. Table I gives the melting points obtained.

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[1] Virtanen, A. I., and Karstrom, H., *Acta chem. Fennica*, 1931, series B, **7**, 17.

[2] Pett, T. B., and Wynne, A. M., *Trans. Roy. Soc. Canada*, 1933, **27**, 119.

[3] Wood, H. G., and Werkman, C. H., accepted for publication, *Biochem. J.*

[4] Wood, H. G., and Werkman, C. H., *J. Biol. Chem.*, 1934, **105**, 63.

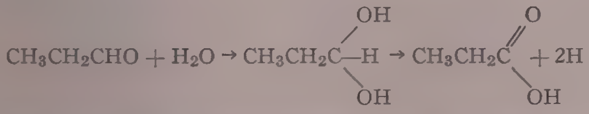
[5] Virtanen, A. I., *Soc. Acta. Fennica, Comment. physico.-math.*, 1923, **1**, 36; 1925, **2**, 20.

[6] Foote, Marion, Fred, E. B., and Peterson, W. H., *Centr. Bakt.*, 1930, 2 abt., **82**, 379.

TABLE I.

	Melting points C°			
Derivative	Unknown	Propion-aldehyde	Mixed	Literature
2,4-dinitrophenylhydrazone	154.5	155.5	155.0	155.0[8]
p-nitrophenylhydrazone	125.0	124.5	124.5	125.0[9]
Dimedon derivative	155.5	155.0	155.0	—

This identification of propionaldehyde formed during the dissimilation of glycerol by *P. arabinosum* strongly suggests its occurrence as an intermediate product. Propionic acid is probably formed by the hydration of the propionaldehyde followed by dehydrogenation.



$$CH_3CH_2CHO + H_2O \rightarrow CH_3CH_2C\begin{matrix}OH\\ -H\\ OH\end{matrix} \rightarrow CH_3CH_2C\begin{matrix}O\\ \\ OH\end{matrix} + 2H$$

The production of propionaldehyde in the fermentation of glycerol has been confirmed by identification of the dimedon (dimethyl-dihydroresorcinol) compound obtained from a fermentation by adding dimedon directly to the medium as a fixative. The medium consisted of yeast extract 0.5%, glycerol 2.0%, $NaHCO_3$ 0.5%, and dimedon 0.33%. Five per cent (by volume) of inoculum of *Propionibacterium arabinosum* was used.

Our attempts to identify propionaldehyde from glucose fermentations have not been decisive. A small amount of precipitate is noted in fermentations, containing dimedon. A purified product has not been obtained as yet from these fermentations. Considering the results in glycerol fermentations, in the absence of contrary evidence, it is reasonable to assume that propionaldehyde is an intermediate in the fermentation of glucose.

From a consideration of the intermediate products which have been identified it appears probable that glucose is broken down in the following way: The first conversion is into a hexose-mono-phosphate which in turn is split into 2 triose-molecules; probably methylglyoxal results. Part of the methylglyoxal is then oxidized to acetic acid and CO_2 by dehydrogenation as suggested by van Niel[10] and Wood and Werkman.[3] Hydrogen arising from this oxidation serves in the reduction of methylglyoxal to α-hydroxy-propionaldehyde and its reduction to propionaldehyde. The conversion of this latter compound to propionic acid occurs as described above. It is of course not proven that propionaldehyde does occur normally in the propionic acid fermentation. It may be formed only under abnormal conditions. For example, fixation of α-hydroxy-propionaldehyde might lead to the formation of propionaldehyde by blocking the production of lactic acid.

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[7] Peterson, W. H., and Fred, E. B., *J. Biol. Chem.*, 1920, **44**, 29.

[8] Allen, C. F. H., *J. Am. Chem. Soc.*, 1930, **52**, 2955.

[9] Bauer, Hugo, and Strauss, Ed., *Ber. deut. chem. Ges.*, 1932, **65**, 308.

Although α-hydroxy-propionaldehyde has not been identified in the propionic acid fermentation, the occurrence of an α-hydroxy intermediate compound is supported by the fact that under certain conditions the authors have been able to demonstrate considerable quantities of lactic acid in glucose fermentations. It is acceptable that lactic acid is formed by hydration and subsequent dehydrogenation of α-hydroxy propionaldehyde. It is reasonable to assume, since lactic acid is fermented by the propionic acid bacteria, that it may also be intermediate in the formation of propionic and acetic acids. This conversion of α-hydroxypropionic acid or lactic acid to propionic acid also lends direct support to the proposed dissimilation of α-hydroxypropionaldehyde to propionaldehyde.

It is reasonable to accept that propionic acid is formed by more than one series of reactions, namely, through the intermediates lactic acid and propionaldehyde, and possibly according to van Niel's scheme.[10]

7383 C

The "Fat-Metabolism" Hormone and Hyperglycemia.

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The finding of Anselmino and Hoffman,[1, 2] that a fraction from the anterior pituitary, when injected into rats, markedly increases the acetone bodies in the blood, received support from the work of Funk,[3,4] who, using rats as test animals, showed that this peculiar "fat-metabolism" hormone is present in the urine of pregnancy and in normal urine. Funk records large increases in urinary acetone bodies. Katzman and Doisy,[5] using their method for getting the gonadotropic factor of pregnancy urine, find that hyperglycemia is produced by these extracts. They worked with rabbits.

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[10] Van Niel, C. B., *Dissertation,* Delft, 1928.

[1] Anselmino and Hoffman, *Klin. Woch.,* 1931, **10**, 2380.

[2] Anselmino and Hoffman, *Klin. Woch.,* 1931, **10**, 2383.

Believing that Funk, on the one hand, and Katzman and Doisy, on the other, were working with an extract which contained the same active material, we prepared our extracts according to Funk's method and tested them on rabbits for blood-sugar, according to Katzman and Doisy. In addition, we also determined acetone bodies in the blood. The results are presented in Tables I and II.

TABLE I.
Glucose.

Preparation No.	Urine equivalent of extract injected (Liters)	Initial blood sugar (Mg. %)	Maximum blood sugar (Mg. %)	% Increase	Time elapsed before maximum value (Hrs.)
1	3	126	353	180	3
2	1.95	94	144	53	6
3	1.8	144	180	25	3
4	3	98	155	58	3
5	3	84	140	67	2
6	3	105	322	207	3
7	2.4	112	133*	19	½
8	3	90	165	83	1

*Rabbit died at the end of the first hour after injection.

TABLE II.
Acetone.

Preparation No.	Urine equivalent of extract injected (Liters)	Initial blood acetone (Mg. %)	Maximum blood acetone (Mg. %)	% Increase	Time elapsed before maximum value (Hrs.)
3	1.8	35.42	135.24	282	2
4	3	9.66	16.9	75	¼
5	3	8.1	16.1	99	2
6	3	12.9	27.4	112	2
7	2.4	32.2	61.18*	90	½
8	3	19.32	74.06	283	1

*Rabbit died at the end of the first hour after injection.

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[3] Funk, C., *Biochem. J.*, 1932, **26**, 619.

[4] Funk, C., *Proc. Am. Soc. Biol. Chem.*, 1933, **8**, 43.

[5] Katzman, P. A., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 315.

Not only do we get hyperglycemia comparable to that obtained by Katzman and Doisy, but we also get a very definite increase in the acetone in the blood. We also get a decrease in CO_2-combining power and an increase in lactic acid. We may add that, aside from one result which appears anomalous, all attempts so far to obtain appreciable increases in urinary glucose and acetone have failed.

It is, perhaps, somewhat premature to interpret the significance of these results. However, as a tentative hypothesis, we may regard this anterior pituitary-like substance eliminated in the urine as being involved in carbohydrate metabolism, primarily, and in fat metabolism, secondarily.

The method of preparing the extract is as follows: To 3 liters of urine (we have used the urine of males from the ages 18-22) are added, very slowly, and with violent stirring, 90 gm. of benzoic acid dissolved in a minimum quantity of alcohol. The product is filtered and the precipitate is suspended in a volume of alcohol equal to the amount used for dissolving the benzoic acid. After considerable stirring at room temperature, the material is centrifuged, the supernatant liquid poured off, and the residue again suspended in alcohol —this time using one-half the original volume. The product is centrifuged. The supernatant liquid is poured off and ammonia is added to the residue until the suspension reacts just pink to phenolphthalein. The suspension is centrifuged and the supernatant liquid, which is the required extract, is poured off. The final extract should not be used unless it is at a pH 8-9. Our extracts are so prepared that 10 cc. of the final product is equivalent to 3 liters of urine.

7384 P

The Velocity of Blood Flow in Part of the Pulmonary Circulation.

H. R. MILLER. (Introduced by H. O. Mosenthal.)

From Montefiore Hospital, New York City.

It is possible to time the velocity of the blood current in man, starting, for example, from the median basilic vein in the elbow and ending at the alveoli of the lungs. The intervening distance may be considered as a constant circuit, part of the pulmonary circulation. The time needed for blood to traverse this intervening distance was carefully noted in conditions of health and disease. This report, based on studies in man, describes the technique of the method employed, results obtained by this method, and its application in physiological and clinical problems.

The subject is recumbent in bed. His mouth having been freely rinsed with tap water, directly before the test, he is instructed to breathe rapidly, about 40 excursions per minute, and to hold his mouth wide open. At ordinary room temperature (70° to 72 F.) and under usual atmospheric conditions (753 mm. Hg.) a measured quantity of a non-toxic substance possessing an odor easily identified is quickly injected, within one second, into the right or left median basilic vein at the elbow and the arrival of the odor in the subject's breath instantly noted. The time required for the odor to complete this circuit is carefully clocked.

For intravenous injections the following substances have been utilized: guaiacol, sodium cacodylate, allyl sulphide, methyl salicylate, ether, and perfumes, more especially ingredients in the preparation of jasmin perfume, benzyl acetate and linalyl acetate. The sharpest results are obtained with perfume and ether. Our earliest use of ether, October, 1932, was suggested by the work of Burkhardt, Kümmell, and Küttner,[1, 2, 3] who published results on the intravenous administration of ether as an anesthetic and commented on the prompt appearance of ether in the breath. At first we injected usually 1 to 1.5 cc. of ether, but at the site of injection thrombosis would be likely to occur, and in some subjects, those with cardiovascular disease, ether seemed to produce acute, transient, rather severe precordial pain. Other substances were therefore tried and the technique modified to eliminate these objectionable features. The more recent tests are free of untoward effect; they have been carried out with the following preparations: (a) 3 cc. of physiological saline solution containing 4% ether by volume, each cc. contains .04 cc. ether, (b) .15 cc. ether with .025 cc. benzyl acetate and .025 cc. linalyl acetate, (c) .5 cc. to 1 cc. of a solution made up of 1 cc. distilled water, .5 cc. benzyl acetate, .5 cc. linalyl acetate.

In normal subjects of both sexes in ages from 16 to 70 years, the average velocity in the majority of cases is about 6 to 9 seconds. Occasionally it is 3.5 seconds and in other cases 11.5 seconds. These figures in the same subjects under similar conditions are always less than the time required for the completion of the general circulation

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[1] Burkhardt, *Arch. Exp. Pathol. u. Pharmakol.*, Bd. 61; *Muench. Med. Wochenschr.*, Nos. 33 and 46, 1909; No. 7, 1910; No. 15, 1911.

[2] Kümmell, H., *Keen's Surgery*, **6**, 976; *Centralbl. d. Chir.*, 37, 1910 (Referat), and 12, 1911 (Referat).

[3] Küttner, *Centralbl. d. Chir.*, 37, 1910.

as estimated by the saccharine method. In pathological conditions, as in normals, the blood flow through the partial pulmonary circuit is completed in about the same time, 6 to 9 seconds. The test was performed in patients with rheumatic, luetic and hypertensive cardiovascular disease free of or associated with various stages of congestive failure, in bronchial asthma, pulmonary neoplasm, nephritis, myxedema, arthritis. In several instances where there was clinical reason to believe that the flow in the lesser circulation was retarded, the rate of speed through this circuit was decreased, *i. e.*, 15 seconds or more.

The test is of use in estimating the speed of blood flow through the pulmonary circulation up to the alveoli. Respiratory changes, voluntary or involuntary, may be able to influence this speed. No data are available on this point. No details are at hand on the influence of changes in atmospheric pressure or of marked variations in environmental temperature. It appears that the rate of speed in this circuit is quite constant, 6 to 9 seconds, and that it remains undisturbed even in marked heart and lung alterations, often when the rate of flow in the general circulation is markedly prolonged. It may therefore be stated that, as a rule, retardation of the general circulation does not involve the rate of blood flow in that portion of the pulmonary circulation measured by the test described above. Retardation of the blood flow over this part of the lesser circulation may be governed by forces functioning independently of the general systemic circulation.

7385 C

Scalp Products and Hair Before Puberty as Culture Medium for Certain Pathogenic Fungi.

JOHN W. WILLIAMS.

From the Homberg Memorial Infirmary, Massachusetts Institute of Technology.

A study of the growth of pathogenic fungi on a mixture of barber shop hair and scalp products of adults was reported.[1] In this paper the results of a study of the growth of pathogenic fungi on the hair and scalp products of children under 12 years, before and after extraction with ether, are reported.

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[1] Williams, John W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 586.

The hair was divided into 2 portions and one portion was extracted with ether for 24 hours in a Soxhlet. Portions of the material were placed in test tubes, moistened with distilled water and autoclaved. Plants were made on the extracted and unextracted hair and Sabourauds' proof medium with the following pathogenic fungi and saprophytes, *Lichtheimia sp.* and *Scopulariopsis brevicaulis; Achorion schoenleinii, Acladium castellani, Candida candida, Endodermophyton tropicale, Endomyces capsulatus, Endomyces dermatidus, Epidermophyton inguinale, Glenospora gammeli, Geotrichum bachmann, Indiella americana, Monilia albicans, Microsporon apiospermum, Microsporon audouini, Oöspora humi, Trichophyton crateriforme, Trichophyton granulosum, Trichophyton japonicum, Trichophyton interdigitale, Willia anomala.*

The results of the observations over a period of 30 days were as follows: Growth on Sabouraud's occurred in all instances within 3 days. Growth on extracted hair occurred in all instances with the exception of *Endomyces capsulatus,* while growth on plain hair was absent only in the case of *Achorion schoenleinii.* Growth was much more pronounced on extracted hair with *Glenospora gammeli, Indiella americana, Microsporon apiospernum, Microsporon audouini, Monilia albicans, Trichophyton interdigitale.* Growth occurred as early as the third day on both extracted and unextracted hair with *Acladium castellani, Candida candida, Geotrichum bachmanni, Indiella americana, Lichthiemia sp., Microsporon apiospermum, Monilia albicans, Oöspora humi, Scopulariopsis brevicaulis, Trichophyton crateriforme, Trichophyton granulosum, Trichophyton interdigitale, Trichophyton japonicum.* Growth on extracted and unextracted hair by the seventh day was noted in the following additional cultures: *Endodermophyton tropicale, Epidermophyton inguinale, Glenospora gammeli, Microsporon audouini, Willia anomala.* Growth with *Endomyces dermatitidus* was noted in 12 days on both extracted and unextracted hair, while growth of *Achorion schoeneinii* was noted in the extracted and of *Endomyces capsulatus* on plain hair only.

In general, it may be said that extracted hair seems to favor growth of the organisms. From the above it is apparent that hair of children under 12 is for a number of pathogenic fungi an excellent culture media. Future papers will give data on hair of men and women; an attempt will be made to further separate hair ingredients.

7386 C

Passage of Ovoglobulins Through the Shell Membrane.

EDMUND McNALLY. (Introduced by T. C. Byerly.)

From the Bureau of Animal Industry, U. S. Department of Agriculture.

It has been shown by Pearl and Curtis[1] that during the sojourn of the egg in the uterus the weight and nitrogen content of the albumen increases by the addition of a nitrogenous solution after the shell membrane has been formed. Data were collected to observe what kind of nitrogenous materials were added after the shell membrane was formed.

Mature eggs to be used as controls were obtained in the morning from hens just beginning to lay a clutch of eggs, so that it was likely that they would lay the next day. Four to 6 hours after the egg was laid the hens were sacrificed and the immature eggs removed from the uterus. At this time the eggs were found with the shell membrane formed. The mature egg of the morning and the later immature egg from the same hens were analyzed as pairs.

The eggs so obtained were separated into yolk, thick albumen and thin albumen. A screen, similar to that described by Holtz[2] and Almquist was used for the separation of the thick from the thin albumen. Thirteen pairs of eggs were separated in this manner. The results of the separation of the albumen and the yolk were found to confirm Pearl and Curtis in that only 60% of the albumen by weight, and 90% of the nitrogen in the albumen were present after the shell membrane was formed, and it was for the most part thick albumen.

TABLE I.
Average Weight of Parts of Eggs.

	Yolk	Albumen Thick	Albumen Thin
	gm.	gm.	gm.
Mature eggs	17.67	20.13	13.69
Immature ”	17.38	17.97	2.35

The albumens were diluted, the ovomucins were removed with the centrifuge, and the supernatant fluid was made up to volume with a small amount of sodium chloride or sodium sulfate present to prevent precipitation of the ovoglobulins. The ovoglobulins and the ovalbumins were separated by precipitation of the ovoglobulins with

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[1] Pearl, R., and Curtis, M. R., *J. Exp. Zool.*, 1912, **12**, 99.

[2] Holtz, W. F., and Almquist, H. J., *Hilgardia,* 1931, **6**, 49.

1.5 M sodium sulfate by a technique similar to that of Howe[3] for blood serum proteins. Non-protein nitrogen was determined by using Alman's tannic acid reagent or 2.12 sodium sulfate for protein precipitation. The ovomucoid content was determined by heat coagulation of the other proteins in the presence of acetate buffer approximately pH 4.7 and 0.2 M sodium sulfate. No attempt was made to separate the ovoglobulins or ovalbumins. Nitrogen was determined by macro-Kjeldahl.

TABLE II.
Average Protein Distribution in Ten Eggs.

	Ovomucin N mg.	Ovoglobulin N mg.	Ovalbumin N mg.	Ovomucoid N mg.	Non-protein N mg.
Mature eggs	42.1*	45.38	350.19	61.55	17.48
Immature eggs	40.4*	15.44	342.58	53.02	16.64

* Average of 5 eggs only.

The ovoglobulin fraction shows the only significant difference and in this case, in each of the 10 pairs, the mature egg contains the larger amount. The material added through the membrane is a dilute solution of ovoglobulin with perhaps the salts necessary to keep it dispersed. The passage of the ovoglobulin through the shell membrane shows a behavior similar, in some respects, to that noted by Howe[4] in the rapid passage of globulins into the blood stream of young animals.

7387 P

Immunological Specificity of Carbohydrates Derived from Staphylococci.*

L. A. JULIANELLE AND C. W. WIEGHARD.

From the Bacteriological Laboratories of the Oscar Johnson Institute, Washington University, School of Medicine, St. Louis.

During the course of studies concerning hypersensitiveness in patients with trachoma, it became necessary to determine the possibility of the existence of different immunological types among Staphylococci. Preliminary experiments undertaken with the agglutination reaction revealed that strains isolated from different sources and conditions are not differentiated satisfactorily by this technique. Consequently an effort was made to fractionate carbohydrates from this organism, and this was accomplished successfully.

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[3] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 93.

[4] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 115.

* Conducted under a grant from the Commonwealth Fund of New York City.

The method finally adopted for the separation of carbohydrates from Staphylococci may be outlined as follows: (1) concentration of broth cultures (18-24 hours) by centrifugation in a Sharples; (2) heating the thick suspension in N/16 HCl for 20 minutes in a boiling water bath; (3) after centrifuging, the supernatant is treated with 40% NaOH until flocculent precipitation occurs, which is at a slightly acid reaction; (4) to the supernatant N acetic acid and Na acetate, and 2 volumes of 95% alcohol are added; (5) after standing in refrigerator over night, the residue following centrifugation is taken up in H_2O, and the supernatant is reserved for further separation; (6) the water solution is treated with N acetic acid until precipitation occurs and to the supernatant after centrifugation are added 2 volumes of 95% alcohol and sufficient Na acetate to cause precipitation. The residue is re-extracted until all the soluble material is obtained; (7) up to 6 precipitations with acetic acid are sometimes required to cause complete removal of precipitable matter. The end product never precipitates in the usual protein precipitants; it is always ninhydrin negative, although from time to time biuret positive. The total nitrogen has varied up to 5%. The Molisch reaction is always strongly positive.

In testing the reactivity of this material in rabbit antisera, it was found that while all sera contain a high titre of agglutinins following immunization to Staphylococcus, only about one-third possess precipitins for this material. Where precipitation occurs, the titres are high reaching one to 6 or 8 million. On the basis of precipitation in antisera, then, it was shown that at least 2 serologically type-distinct substances occur in Staphylococci. The one has been found always and only in strains isolated from pathologic conditions, and the other, always and only in saprophytic strains, regardless of source. Thus far 15 strains have been studied by this method. Of these, 9 isolated from different human infections have fallen into one type, while the remaining, isolated from non-pyogenic conditions have fallen into a second type. It is, therefore, proposed to designate the virulent, pyogenic strains as Type A and the avirulent, non-pathogenic strains as Type B.

Both materials appear to be carbohydrates and should be termed soluble specific substances as originally described in Avery's laboratory. While the chemical characteristics are still in the course of study, sufficient data are available to indicate that the 2 substances are carbohydrates as chemically distinct as they are serologically. Because of the preliminary nature of the data, however, they are being reserved for more complete study.

In contrast to the type-specificity of the carbohydrate, is the species-specificity of the "nucleoproteins" derived from the organisms. While the proteins are obviously mixtures, it is nevertheless clear that these solutions react in common in homologous and heterologous antisera.

It is interesting to point out a recent observation in 2 patients, one ill with a Staphylococcus septicemia, the other with osteomyelitis due to the same organism. With convalescence both patients exhibited a marked skin reactivity to 0.1 cc. of a 1-50,000 dilution of the carbohydrate of Type A, but not Type B. Coincident with the development of heightened skin reactivity, the blood serum of the patients showed a high titre of precipitins for the homologous carbohydrate.

7388 P

Effect of Irradiation of Hypophysis on Experimental Diabetes.

W. A. SELLE, J. J. WESTRA AND J. B. JOHNSON. (Introduced by M. Bodansky.)

From the Departments of Physiology and Radiology, The University of Texas Medical School.

Various investigators have presented evidence to show that the hypophysis plays an important rôle in carbohydrate metabolism. The first conclusive evidence is that of Houssay and coworkers, who found (1) that extracts of the anterior lobe of the hypophysis injected into normal dogs produce hyperglycemia, glycosuria and ketonuria, and when injected into depancreatized dogs, an increase in severity of the diabetes;[1, 2] (2) that removal of the hypophysis in normal dogs leads to a hypersensitivity to insulin,[3] hypoglycemic convulsions during fasting, and an increased tolerance to glucose;[4, 5] (3) that in depancreatized dogs, removal of the hypophysis restores the carbohydrate metabolism to an approximately normal condition,

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[1] Houssay, B. A., and Biasotti, A., *Rev. Soc. Argent. Biol.*, 1931, **7**, 3.

[2] Houssay, B. A., *Klin. Wchnschr.*, 1933, **12**, 773.

[3] Houssay, B. A., and Magenta, M. A., *C. R. Soc. Biol.*, Paris, 1925, **92**, 822.

[4] Braier, B., *Rev. Soc. Argent. Biol.*, 1931, **7**, 140.

[5] Houssay, B. A., and Biasotti, A., *Endocrinology*, 1931, **15**, 511.

but renders the animal hypersensitive to insulin;[6, 7] that injection of active anterior-lobe extracts causes a reappearance of the symptoms of diabetes in hypophysectomized-depancreatized dogs.[8] These findings have been largely confirmed by the work of Evans *et al.*,[9] Barnes and Regan,[10] Daggs and Eaton,[11] and Corkill *et al.*[12]

Since the removal of the hypophysis decreases the symptoms of experimental diabetes, it was thought that destructive doses of X-ray to the hypophysis might produce similar results.

Six dogs were depancreatized and allowed to recover under insulin administration. About one week after operation they were placed on a basic diet of dog biscuits, cane sugar and freshly ground meat, to which was added liberal amounts of fresh or dried pancreas. Approximately 2 units of insulin per kilo body weight were given twice daily at the time of feeding. After the wound had healed completely, usually within 3 weeks, food and insulin were withheld for a period of 18 to 20 hours, and the tolerance of the animal to orally administered glucose was determined. After taking an initial blood sample, 1.75 gm. glucose per kilo was given by stomach tube, and further blood samples were taken at 15, 45, 75, and 105 minute intervals. Blood sugar was determined by Folin's method.

Following one or more control glucose tolerance tests, the animals were treated with varying doses of X-rays. During the treatment the animals were anesthetized with nembutal, and tied to a support which held the head securely in any desired position. In the irradiation, 140 kilovolts with 5 milliamperes were used at a distance of 40 to 50 cm., with one-half mm. copper and one mm. aluminum filtration. The rays were directed to the hypophysis through one dorsal and 2 lateral ports, obtaining a cross-firing effect. The duration of treatment varied from 90 to 240 minutes for each port. In 3 of the animals treatment was repeated after 3 weeks with a larger dose. The total duration of irradiation per port for the 6 animals was 100, 180, 90 plus 180, 120 plus 180, 130 plus 240, and 240 minutes respectively. Epilation occurred 3 weeks after irradiation in all ani-

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[6] Houssay, B. A., and Biasotti, A., *Rev. Soc. Argent. Biol.*, 1930, **6**, 251.

[7] Houssay, B. A., and Biasotti, A., *Pfluger's Archiv. ges. Physiol.*, 1931, **227**, 664.

[8] Houssay, B. A., Biasotti, A., and Rietti, C. T., *Rev. Soc. Argent. Biol.*, 1932, **8**, 469.

[9] Evans, H. M., Meyer, K., Simpson, M. E., and Reichert, F. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 857.

[10] Barnes, B. O., and Regan, J. F., *Endocrinology*, 1933, **17**, 522.

[11] Daggs, R. G., and Eaton, A. G., *Am. J. Phys.*, 1933, **106**, 299.

[12] Corkill, A. B., White, H. P., and White, W. E., *J. Physiol.*, 1933, **80**, 193.

mals which received a minimum of 130 minutes per port. Following irradiation, glucose tolerance tests were made weekly for periods of 3 to 5 weeks. In no instance did the fasting blood sugar or the tolerance curve indicate that the treatment had been beneficial within this period. The fasting blood sugar remained between 300 and 600 mg. %, the animals did not become hypersensitive to insulin as do hypophysectomized-depancreatized dogs, and the insulin dosage was not reduced.

This work is being continued with larger doses of X-rays, and a histological study is being made of the pituitary and other irradiated areas of the brain, and also of the thyroid, adrenals, and gonads to determine the effects of the irradiation.

We are greatly indebted to E. R. Squibb and Sons and to Sharpe and Dohme for donating part of the insulin used in this investigation.

7389 P

Effects of Brain Cautery on Fetal Development in the Rat.

E. L. COREY. (Introduced by H. E. Jordan.)

From the Physiological Laboratory, University of Virginia Medical School.

The primary purpose of this study is to determine whether the fetus can survive extensive damage to the brain, and the possible effects of brain injury on subsequent growth and development. Previous studies have been almost entirely confined to observations on teratological (anencephalic) human material (Preyer,[1] Bruns *et al.*,[2] Nañagas,[3] Marcus and Nickman[4]).

Destruction of portions of the fetal brain (rat*) was accomplished by means of the electric cautery. No repair was found necessary other than suture of the maternal body wall (Nicholas[5]). Fetuses recovered from the uterus or taken at birth subsequent to such procedure were examined as to behavior, measured, weighed and placed at once in 5% formalin preparatory to microscopic study of the injured area. Unoperated litter-mates served as controls. A series of control experiments involved (a) injection of carbon ink into the ventricles of the brain, and (b) extensive cautery of an extremity. Seventy fetuses from 21 litters comprise the experimental material, and 36 fetuses from 10 litters were used in the control experiments.

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[1] Preyer, W., *Specielle Physiologie des Embryo,* 1885.

[2] Bruns, I., Cranen, A., and Ziehen, Th., *Hand. d. Nerven. Krankh. im Kindesalter,* Berlin, 1912.

[3] Nañagas, J. C., *Am. J. Anat.,* 1925, **35**, 455.

[4] Marcus, J. H., and Nickman, E. H., *Arch. Pediat.,* 1930, **47**, 739.

* The rats used in this study were obtained through the generosity of the Wistar Institute of Anatomy and Biology.

[5] Nicholas, J. S., *Anat. Rec.,* 1925, **31**, 385; PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 436.

Of the 70 operated fetuses ranging between 11 and 37 mm. of crown-rump length at operation, 40 survived until term or were recovered alive from the uterus 1, 2, 3, 4, 5, or 6 days following cautery. The remainder were either aborted (2) or partially resorbed. At term (39 to 42 mm.) 11 fetuses died *in utero* and none of the animals survived parturition more than 24 hours. The rat fetus may survive brain cautery until term regardless of the stage of development at which the operation is performed.

Living operated fetuses recovered from the uterus or those born dead or alive present certain distinct gross abnormalities (aside from cranial malformations attributable to cautery *per se*): an enlarged and protruding tongue, thickened body contour with heavy neck and jaw and an abnormally shortened rostral region. The maxillae are more markedly affected than the mandibles. There is little if any repair of the injured area although the general vascularity appears unimpaired.

All living fetuses recovered from the uterus or those born alive exhibit spontaneous (*i. e.,* non-specifically induced) movements indistinguishable from those shown by their litter-controls. The application of tactile stimuli by bristle or needle elicits no marked difference in response when applied to operated and control fetuses from the same litter, although the strength of the response appears weakened and the reactions sluggish in the operated fetuses. These results indicate that the cerebral cortex of the fetus cannot be extensively involved in the production of fetal movements either spontaneous or induced. It is suggested, however, that the cortex may be necessary for (quantitatively) normal movement. Both operated fetuses and their litter-controls show vigorous respiratory movements and the ability to squeal.

Crown-rump length and weight were selected as criteria for a comparison of the rate of development of the operated fetuses with that of their litter-controls. It was found that the operated fetuses average 8% below the controls in crown-rump length and 10% below the normal fetuses in weight. The lesser crown-rump length of the operated animals may possibly be due to the pronounced abnormality of the head region, and the figure obtained (—8%) is perhaps of little significance. The relatively small difference (—10%) in body weights may likewise be of only slight significance since the apparent retardation in growth shown by the cauterized fetuses could be attributed to tissue lost at operation, together with faulty development in the region of cautery. Nevertheless, the fetuses showing the greater weight discrepancy when compared with their litter-controls are those operated at the earlier stages. Thus litters 1, 8 and 16, operated at crown-rump lengths of 12, 11 and 10 mm. respectively, show smaller body weights when compared with their litter-mates by 25, 24, and 23%. That these are the youngest fetuses operated, and that they show the most pronounced apparent retardation of development may not be wholly without significance. The results are summarized below.

Number of litters used, 21. Number of fetuses operated, 70.
Days in uterus after operation: range, 1 to 6; average, 4.
Operated fetuses surviving until term, 40.
Crown-rump length of operated fetuses at operation: range, 11 to 37 mm.; average, 21 mm.
Crown-rump length of operated fetuses on recovery from uterus. range, 21 to 41 mm.; average, 36 mm.
Crown-rump length of control fetuses on recovery from uterus: range, 25 to 42 mm.; average, 39 mm.
Weight of operated fetuses on recovery from uterus: range, 1.1 to 5.7 gm.; average, 3.8 gm.
Weight of control fetuses on recovery from uterus: range, 1.4 to 5.3 gm.; average, 4.3 gm.

Microscopic study of the material reveals unilateral and bilateral destruction of the hemispheres with extensive thalamic and cerebellar injury in certain cases and section of the brain-stem at various levels above the medulla in others. No correlation could be demonstrated between post-operative survival, behavior or rate of growth and the size and location of the areas destroyed, provided the medulla remained uninjured.

The control experiments gave wholly negative results. It is interesting to note that no instances of hydrocephalus occurred following the injection of carbon ink into the brain ventricles (Weed[6]), although the presence of the ink is discernible in histological section in all cases.

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[6] Weed, L. H., *Contr. Emby. Carnegie Inst. Wash.*, 1920, No. 44, 425.

7390 C

Respiration of Yeast in Water Containing Deuterium Oxide.

G. WELLFORD TAYLOR* AND E. NEWTON HARVEY.

From the Physiological Laboratory, Princeton University.

Harvey and Taylor[1] have shown that concentrated heavy water markedly inhibits the respiration of 2 species of luminous bacteria and diminishes the luminescence of one form *(Bacillus fischeri)*. The effect on the respiration of *Bacillus fischeri* was such that if the curve relating effect on respiration with concentration of heavy water is extrapolated, zero respiration is indicated in 100% heavy water. Reduction of respiration of *Vibrio phosphorescens* was not so great.

Experiments with yeast indicate that the effect of heavy water on respiration of this organism is also not so great. These are detailed below together with a study of the respiration in as near 100% heavy water as is experimentally feasible considering the hygroscopic properties of D_2O. Pacsu[2] has reported a 9-fold reduction of yeast fermentation (CO_2 production) in approximately 100% D_2O and 1.6 reduction in 60% D_2O.

The heavy water was provided by Professor H. S. Taylor, and had been prepared by electrolysis and burning of deuterium and oxygen in the manner described by Taylor, Eyring and Frost.[3] It had also been distilled from alkali and permanganate. High concentrations of such water is toxic for paramoecium, whereas low concentrations, (0.2%) although prepared in exactly the same manner and also distilled from alkali and permanganate in the same vessel, are not toxic, so that the diminution of respiration of cells in concentrated heavy water must be connected with the deuterium which it contains and *not* with impurities of any kind. Yeast in contact with 0.2% D_2O for 17 hours and control yeast in contact with distilled water for 17 hours both respire at the same rate (within 5%) when nutrient is added, showing that no deleterious impurities having a cumulative effect were present.

For experimental purposes a cake of Fleischmann's yeast was suspended in 50 cc. of nutrient medium and aerated vigorously for several hours to insure an even distribution of cells. Since nutrient is necessary to give an even rate of respiration, 1 cc. portions of Williams medium were evaporated to dryness and to the residue were added 1 cc. portions of (a) concentrated heavy water, (b) ordinary distilled water, and (c) 0.2% heavy water. These solutions were placed in Warburg respiration chambers and then 1 drop of the yeast suspension was very slowly dropped from a special pipette into each chamber. Experiments were always performed in pairs and the pairs measured at the end of one hour, agreed to within ±3%. The distilled water and 0.2% D_2O respired at the same rate, within 3%, the 0.2% D_2O respiring 3% more rapidly.

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* National Research Fellow in Biology.

[1] Harvey, E. N., and Taylor, G. W., *J. Cell and Comp. Physiol.*, 1934, **4**, 357; *Biol. Bull.*, 1934, **66**, 91.

[2] Pacsu, E., *J. Am. Chem. Soc.*, 1934, **56**, 245.

[3] Taylor, H. S., Eyring, H., and Frost, A. A., *J. Chem. Physics*, 1933, **1**, 823.

The procedure with 100% D_2O was slightly different. In order to maintain a concentration near 100%, a drop (.05 cc.) of yeast suspension was added as above to each of 2 tubes of 1 cc. of 100% heavy water, and this allowed to stand for 15 minutes in a desiccator.

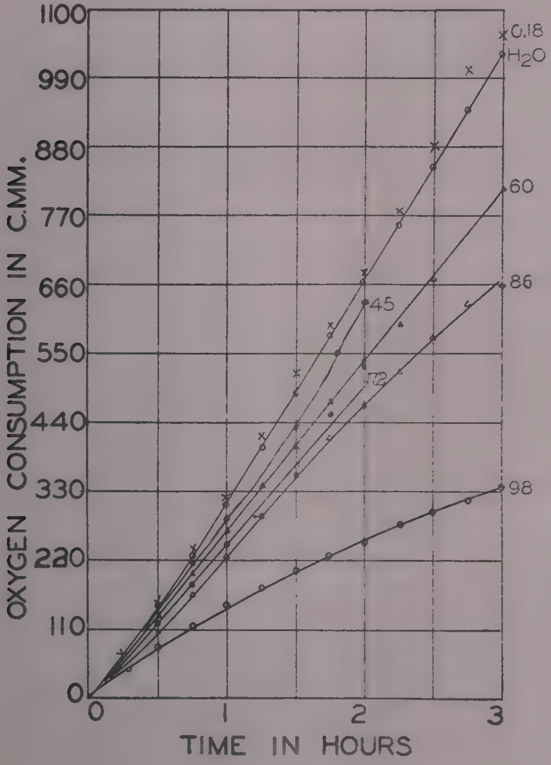

Fig. 1.

They were then centrifuged, the supernatant fluid being poured off and fresh 100% heavy water containing nutrient added to the yeast. Two controls with ordinary distilled water were treated in exactly the same way. Then the samples were placed in the Warburg apparatus and their rate of respiration at 25°±0.01°C. determined. In this experiment instead of using aqueous KOH to absorb CO_2, 100% heavy water to which metallic sodium had been added, forming NaOD, was placed in the vessels so that no H_2O could distill over from the alkaline solution. Ordinary water was used in the manometers since experiments with dry blue cobalt chloride paper† and glycerine as a hygroscopic material showed that water vapor diffuses only slowly (1 hour) from the manometer fluid but rapidly (10 min.) from aqueous alkali in the respiration chambers. Although the day on which the experiment was performed was bright and clear there was no doubt some unavoidable absorption of moisture during handling of the D_2O, and the final concentration might be placed at 97-98%.

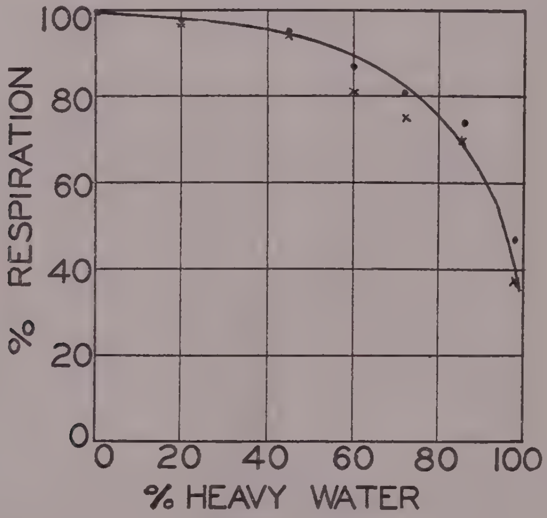

FIG. 2.
Dots show % respiration after one hour; crosses after 2 hours.

Results are best expressed by reference to Fig. 1, showing the oxygen consumption with time when the yeast was in various concentrations of heavy water, and by Fig. 2 showing the relation between rate of respiration and per cent concentration of heavy water. It will be observed that concentrations below 20% had no inhibitory effect, 45% caused a 5% inhibition, 60% about 13%, 72% heavy water about 20%, 86% heavy water about 27%, and 97-98% heavy water about 50% inhibition after one hour. The effect tends to increase after 2 hours. If respiration is to be reduced to zero in 100% heavy water the curve must be very steep between the values 80% and 100%, a point difficult to test on account of the hygroscopic properties of D_2O.

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† This paper becomes pink on absorbing water.

7391 C

Effect of Combination of Two Antagonistic Anterior Pituitary Hormones on Sex Organs of Guinea Pig.*

HELEN M. AFF and LEO LOEB.

From the Department of Pathology, Washington University School of Medicine, St. Louis.

It has been shown that 2 antagonistic hormones are present in the anterior pituitary gland, one causing growth and maturation of follicles and the other atresia of follicles in the ovary of the guinea pig; the former predominates in the anterior pituitaries of rabbit, rat and guinea pig and the latter in the glands of cattle, sheep and hog, at least in the state in which these latter are accessible to us for experimentation.[1, 2, 3] When as the result of the action of the first hormone the follicle has reached full size and matured, either ovulation and formation of true corpora lutea may take place or there may be ingrowth of vessels and connective tissue into the granulosa and formation of pseudocorpora lutea not associated with ovulation.[2] There occurs a third effect common to the substances in which both of these hormones are found, a transformation of theca interna, and probably also of connective tissue cells into strands of interstitial gland and into interstitial gland bodies, which transformation is found in the course of follicular atresia.[4] Tran-

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* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, and Friedman, Hilda, Proc. Soc. Exp. Biol. and Med., 1933, **30**, 741.

[2] Loeb, Leo, *Ibid.*, 1933, **30**, 1335.

[3] Loeb, Leo, *Ibid.*, 1932, **29**, 642.

[4] Loeb, Leo, *Endocrinology*, 1932, **16**, 129.

sitions from the interstitial gland bodies to small pseudocorpora lutea occur and such pseudocorpora lutea may be seen especially after several injections of extract of cattle anterior pituitary into the guinea pig. Smaller amounts of pseudolutein tissue may furthermore be produced in the ovary of the guinea pig in follicles undergoing connective tissue atresia as a result of cattle anterior pituitary extracts. However, we do not wish to exclude the possibility that under certain conditions also granulosa may participate in the formation of these structures, at least in a limited way.

Under these conditions it was of interest to study the combined effect of these 2 antagonistic principles in the guinea pig. Such investigations were begun several years ago; at that time we combined injections of extract of cattle anterior pituitary with implantations of guinea pig anterior pituitary and in one case with implantation of anterior pituitaries of rabbit.[4] We found an inhibiting effect of the action of the extract on the growth promoting action of rabbit and guinea pig anterior pituitary. Continuing these experiments we wished to determine whether the atresia-causing hormone of the anterior pituitary would still be able to counteract the hormone causing follicular growth and maturation, in case the extract of cattle anterior pituitary containing the former principle were given in a first period and this were followed in a succeeding period by daily inoculations of either guinea pig, rat or rabbit anterior pituitary glands. In control experiments, the guinea pigs in a first period were underfed so that they lost weight like those animals which had been injected with cattle anterior pituitary extract. In addition the effects of inoculation of various types of anterior pituitary glands in well fed guinea pigs, as well as the effect of underfeeding alone and of extract not accompanied by implantation of anterior pituitary glands, were tested. The numerous experiments, which we carried out were varied as to the quantities of both kinds of anterior pituitary material used and as to the length of time during which these hormones were applied.

We used in our experiments guinea pigs, because, as we have pointed out previously, the ovary of this animal represents the most delicate reagent for the 2 principles present in the anterior pituitary glands of various species, the one causing atresia of follicles and the other promoting the development of mature follicles; in the rat, which has usually served as test animal for investigators who studied the effect of combinations of different anterior pituitary preparations or of anterior pituitary-like preparations, the atresia producing effect of certain anterior pituitary preparations cannot be discovered or it can be distinguished from other effects only with great difficulty.[5]

The initial weight of the female guinea pigs, used in our experiments, varied between 175 and 220 gm. We used the acid extract of cattle anterior pituitary to which reference has been made in our earlier publications[6] and which has served for the large majority of our experiments; however, as we have pointed out previously, alkali extract, and as we may add, extract prepared with 0.9% NaCl solution, act in principle in the same way. The anterior pituitary glands which we employed for implantation were those of male animals (rabbits weighing between 1500-2000 gm., rats weighing about 150 gm., and guinea pigs weighing 350-420 gm.).

In a first period of 5 days, the guinea pigs received daily injections of 1 or 2 cc. of extract; or they were underfed, or still others were kept under normal conditions. In a subsequent period varying between 4 and 7 days, the same animals received daily implantations of the anterior pituitary glands, others which did not receive these implantations, served as controls. Fourteen guinea pigs received anterior pituitary glands of guinea pigs (1 or 2 glands daily); 27 guinea pigs were daily implanted with rabbit anterior pituitaries (1/3 to 1 gland each time) and 18 guinea pigs received daily implantations of rat anterior pituitaries (1-2 glands each time).

Forty guinea pigs received injections of acid extract either alone or in combination with subsequent implantations of rabbit, rat or guinea pig anterior pituitary glands. Forty guinea pigs were underfed and a number of these guinea pigs received subsequently implantations of anterior pituitary glands.

Altogether 93 guinea pigs were studied in this way. At the conclusion of the experiments in each case both ovaries were cut into complete serial sections and many sections through uterus, vagina and cervix and mammary gland were prepared.

These investigations led to the following conclusions: 1. The hormone causing atresia of follicles, administered in a first period, prevents the full effect of the hormone causing growth and maturation of follicles administered in a subsequent period.

2. This inhibiting effect is in the same direction as the effect exerted by underfeeding, but it is much more pronounced.

3. The pseudocorpora lutea, which may be produced in the

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[5] We shall discuss the literature concerning the effects of the interaction of various sex hormones on the sex organs in another connection.

[6] Loeb, Leo, and Bassett, R. B., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 860; and 1930, **27**, 490.

first period of extract injection, may still remain well preserved through the second period, during which the follicular growth hormone acts, but in other cases they may perhaps undergo degenerative changes similar to those observed in true corpora lutea during the process of retrogression.

4. There is no antagonism between the effects of the atresia causing hormone and the process leading to the transformation of atretic follicles into interstitial gland and into pseudocorpora lutea, as far as the latter develop at the expense of the theca interna; these changes are not noticeably inhibited by the first named hormone.

5. This result confirms the previous conclusion that the 2 kinds of lutein tissues, one developing from the granulosa and the other from theca interna and probably also from connective tissue cells situated in the medulla and in atretic follicles may owe their development to different kinds of hormone action. Both the extract of cattle anterior pituitary and the anterior pituitary gland substance of rabbit, rat and guinea pig seem to have in common this effect on theca interna and connective tissue transforming them to interstitial gland tissue.

6. We may assume that the atresia causing hormone fixes itself to the follicles, remains combined with them for some time and thus inhibits the full development and maturation processes in the follicles, which otherwise would have been induced by the follicular growth hormone, even after the completion of the period of the extract injections.

7. The hormone causing follicular atresia induces indirectly a state of rest in vagina, uterus and mammary gland; it is probably closely associated or identical with the thyroid stimulating hormone.[7] However, in case pseudocorpora lutea develop in the ovaries under the influence of this hormone, we often, though not always, note changes in the mucosa of the uterus indicating a mild state of stimulation. The follicular growth producing hormone induces the release of the follicular hormone and thus causes proliferation of vagina and mammary gland and an oestrous state of the uterus.

8. We could confirm our previous observation that the growth processes elicited in the vagina by the implantation of anterior pituitaries of rabbit and rat remain usually incomplete, although the number of mature follicles and of lutein bodies developing under

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[7] Loeb, Leo, and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 172.

those conditions in the ovaries may be much greater than in normal ovaries of guinea pigs at the time of ovulation. One single mature follicle present at the time of oestrous in the guinea pig ovary rapidly causes marked proliferation and keratinization of the epithelium of the vagina.

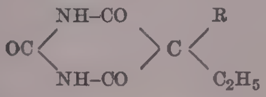
7392 C

Relationship Between Pharmacological Action and Chemical Structure of Barbituric Acid Derivatives.

EDWARD E. SWANSON. (Introduced by K. K. Chen.)

From the Lilly Research Laboratories, Indianapolis, Indiana.

To elucidate the relationship between the chemical structure and the pharmacological action in the barbital series, over 50 derivatives were investigated. These compounds were all 5,5-substituted barbituric acids, having the general formula:



$$\mathrm{OC}\left\langle\begin{matrix}\mathrm{NH{-}CO}\\ \mathrm{NH{-}CO}\end{matrix}\right\rangle\mathrm{C}\left\langle\begin{matrix}\mathrm{R}\\ \mathrm{C_2H_5}\end{matrix}\right.$$

wherein R-alkyl radical (normal or secondary with 2 to 9 C-atoms). Several of them were new compounds synthesized for the first time.[1]

Albino rats weighing from 71 to 126 gm. (average 96 gm.) were employed in this study. Solutions of the sodium salts of the compounds were injected intraperitoneally. The minimal anesthetic dose (M.A.D.), the duration of action, and the minimal lethal dose (M.L.D) were determined by using 5 animals for each dose level.

Since space does not permit a presentation of detailed results, only a few salient points will be discussed here. In Figs. 1 and 2, the "primary alkyls" refer to those compounds having a normal alkyl group. It should be noticed from Fig. 1 that with the increase in number of C-atoms in the alkyl group, either normal or secondary, both the M.A.D. and M.L.D. grow relatively smaller, but when the alkyl radical is longer than 5 C-atoms, the amount required to anesthetize or kill rats again increases. The therapeutic index, or the ratio between the M.L.D. and M.A.D., appears to be gradually greater as the alkyl chain lengthens.

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[1] Shonle, H. A., Waldo, J. H., Keltch, A. K., and Coles, H. W., Read at the Am. Chem. Soc. Meeting, St. Petersburg, Florida, Mar. 25-30, 1934.

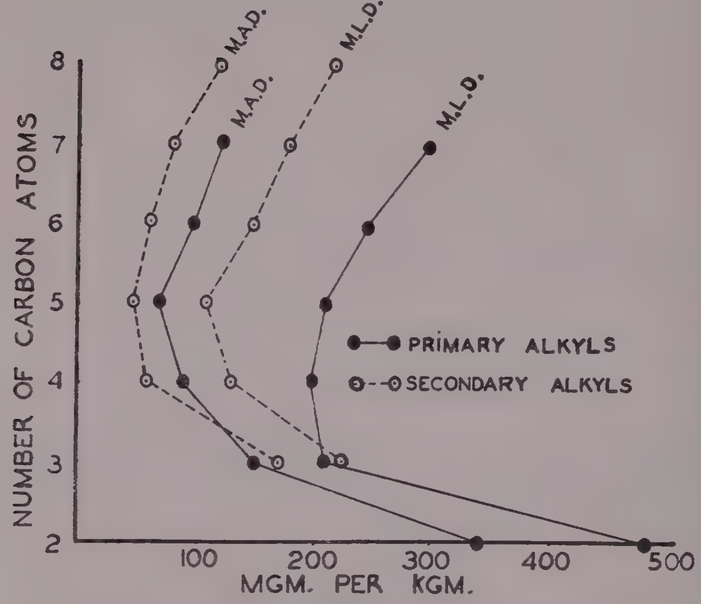

FIGURE I

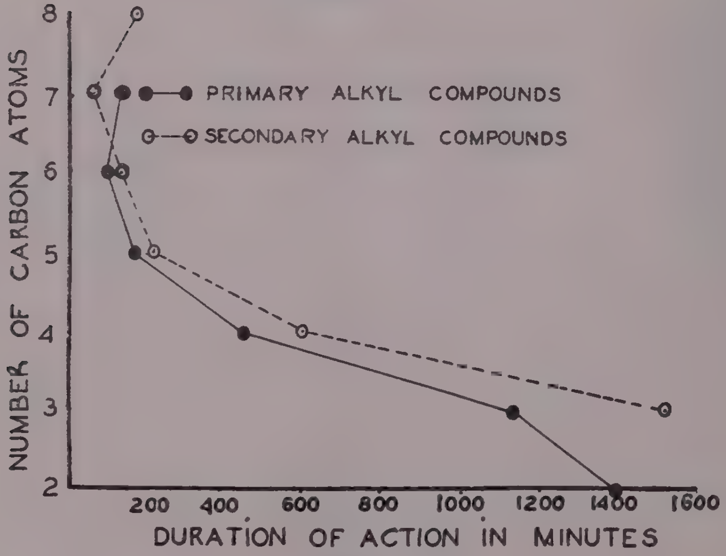

FIGURE II

In general, the duration of action shows similar features; that is, it is shorter when the alkyl group becomes lengthened (Fig. 2). In the series of normal alkyl derivatives, the critical compound is the one that possesses 6 C-atoms at R; and in the series of secondary alkyl derivatives, the critical compound is the one having 7 C-atoms at R, beyond which the duration of action begins to increase.

If R is phenyl, the compound, phenobarbital, has the well-known prolonged action; but if the ethyl group is replaced by a methyl, the resulting substance has a larger M.A.D. and M.L.D., and a shorter duration of action, as compared with phenobarbital.

The author is indebted to Dr. K. K. Chen for criticisms and assistance in the preparation of this manuscript.

7393 C

Short Acting Barbituric Acid Derivatives.

EDWARD E. SWANSON. (Introduced by K. K. Chen.)

From the Lilly Research Laboratories, Indianapolis, Indiana.

In a previous communication,[1] it was pointed out that there is obvious relationship between the pharmacological action and the chemical structure of certain barbituric acid derivatives, and that compounds with long alkyl groups have a shorter duration of action. The present report deals with the evaluation of 12 such compounds, all of which were prepared by Shonle, Waldo, Keltch, and Coles.[2]

Typical hypnotic and anesthetic properties were observed with all the substances except one, injected intraperitoneally, in albino rats. Their minimal hypnotic doses (M.H.D.), minimal anesthetic doses (M.A.D.), and minimal lethal doses (M.L.D.) were determined, and their therapeutic indices calculated as shown in Table I. Compounds numbered 1, 2, 3, and 7 were also tested in dogs, being effective either by vein or by mouth. It is curious that compound numbered 12, 1,3-dimethyl-butyl-ethyl barbituric acid, is devoid of any hypnotic or anesthetic action, but on the contrary, produces convulsions. The substance is also highly toxic.

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[1] Swanson, E. E., PROC. SOC. EXP. BIOL. AND MED. (in press).

[2] Shonle, H. A., Waldo, J. H., Keltch, A. K., and Coles, H. W., Read at the Am. Chem. Soc. Meeting, St. Petersburg, Florida, Mar. 25-30, 1934

TABLE I.

No. of Compound	Barbituric Acid	M.H.D. mg. per kg.	M.A.D. mg. per kg.	M.L.D. mg. per kg.	Average Duration of Action min.	Therapeutic Index: M.L.D./M.A.D.
1	2,4-dimethyl-pentyl ethyl-sodium salt	50	70	140	63	2.00
2	1,4-dimethyl-pentyl-ethyl-sodium salt	60	80	240	69	3.00
3	1-propyl butyl-ethyl-sodium salt	20	30	65	75	2.16
4	1,3-dimethyl-pentyl-ethyl-	50	70	200	102	2.85
5	4-methyl-heptyl-ethyl-	60	80	230	115	2.87
6	2,4-dimethyl-hexyl-ethyl-	60	80	230	132	2.87
7	2-ethyl-hexyl-ethyl-sodium salt	50	80	230	154	2.87
8	4-methyl-pentyl-ethyl-	50	70	180	155	2.57
9	5-methyl-2-ethyl-hexyl-ethyl-	100	140	340	158	2.42
10	2-methyl-pentyl-ethyl-	50	70	180	225	2.57
11	3-methyl-2-ethyl-hexyl-ethyl-	120	160	400	255	2.50
12	1,3-dimethyl-butyl-ethyl-	none*	none*	10*	—	—

*Convulsions.

7394 C

A Procedure for Determining Distribution of Blood Groups in Mummies.*

G. ALBIN MATSON. (Introduced by J. Bronfenbrenner.)

From the Department of Bacteriology, Washington University Medical School, St. Louis.

Some unexpected differences in the distribution of blood groups observed by us a year ago[1, 2] among the "Blackfeet' and "Blood" tribes of American Indians suggested that the different tribes may not have had a single origin and that at least some of the tribes may not have separated from the rest of the human family before the A agglutinogen developed in the race. Since the possibility of a degree of racial admixture among putative full blooded Indians always remains, regardless of their pedigree as given in the Agency records, the desirability of detecting the A agglutinogen in mummies of Indians who lived before the advent of the white man suggested itself.

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* Grateful acknowledgement is made to Dr. J. Bronfenbrenner, who suggested the problem and offered kindly advice and criticism in the development of methods, to Drs. G. D. Williams and H. A. McCordock, who shared with me the mummy material which they secured from the Peabody Museum of Harvard, and to Dr. T. B. Pote, through whose courtesy the cow serum was obtained.

[1] Matson, G. Albin, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1380.

[2] Matson, G. A., and Schroder, H. F., *J. Immunol.*, 1933, **25**, 155.

Through the courtesy of Drs. G. D. Williams and H. A. McCordock, muscle tissue from 12 mummies from Peru, Arizona and Greenland, was placed at our disposal for such a study.

Because of the limited amount of material available in the preliminary experiments, dried cadaver tissues which had been previously washed and dried rapidly under a current of warm air were used in place of the mummy material.

The procedure followed at first was one in which $O(\alpha\beta)$ serum was absorbed with cadaver tissues of groups O and A respectively by allowing mixtures of a small amount of the powdered tissue and varying dilutions of $O(\alpha\beta)$ serum to remain over-night in the refrigerator. After centrifuging, the supernatnant fluid was tested with O, A and B cells to determine whether the absorption of the agglutinins by the tissues had occurred. In such a test it is considered that if the group A cells are not agglutinated while B cells are, that the α agglutinin in the $O(\alpha\beta)$ serum has been absorbed by an A agglutinogen in the tissue; conversely, if B cells are not agglutinated whereas A cells are, the tissue would be considered to have contained the B agglutinogen which absorbed the β agglutinin from the $O(\alpha\beta)$ serum.. If, however, there is no inhibition of agglutination of either A or B cells, it may be assumed that the tissue contains neither A nor B agglutinogen and may consequently be considered to belong to group O.

This procedure gave at times some confusing results, most likely due to the non-specific absorption by the particulate tissue material. To obviate this difficulty attempts were made to extract the isoagglutinogen from the pulverized tissues and to use the extracts rather than the tissues themselves as a source of agglutinogen.

These extracts of cadaver muscle tissue were made by mixing 1 gm. of powdered skeletal muscle tissue with 5-10 cc. of physiological saline solution and subjecting the mixture to repeated freezing and thawing. The tubes were then centrifuged and tests set up with the supernatant fluid. Varying amounts of the supernatant fluid (0.5 cc., 0.3 cc., 0.1 cc., and 0.05 cc.) were mixed with

TABLE I.
Titration of residual agglutinins after absorption of $O(\alpha\beta)$ serum with cadaver and mummy tissues.

Tissue Extract	Serum Dilutions 1:2			1:4			1:8			1:16			1:32		
		Cells			Cells			Cells			Cells			Cells	
	O	A	B	O	A	B	O	A	B	O	A	B	O	A	B
I M 1	—	++++	++++	—	++++	++++	—	++++	++++	—	+++	+++	—	—	—
I M 2	—	++++	++++	—	+++	+++	—	+	+	—	—	—	—	—	—
I M 3	—	++++	++++	—	++++	++++	—	+++	+++	—	++	+	—	+	—
I M 4	—	++++	++++	—	++++	++++	—	+++	+++	—	++	++	—	+	—
I M 5	—	++++	++++	—	++++	++++	—	++++	++++	—	+++	++	—	++	+
I M 6	—	++++	++++	—	++++	++++	—	+++	+++	—	+++	++	—	++	+
I M 7	—	++++	++++	—	++++	++++	—	+++	+++	—	+++	++			
I M 8	—	++++	++++	—	++++	++++	—	+++	++	—	+++	+			
I M 9	—	++++	++++	—	++++	++++	—	++++	+++	—	+++	++	—	++	+
I M 10	—	++++	++++	—	++++	++++	—	++++	++++	—	++++	++++			
I M 11	—	++++	++++	—	++++	++++	—	++++	++++						
I M 12	—	++++	++++	—	++++	++++	—	++++	++++	—	++++	++++			
Cadaver Group O	—	++++	++++	—	++++	++++	—	++++	++++	—	++++	++++			
Cadaver Group A	—	++	++++	—	+	++++	—	—	++++	—	—	+++	—	—	+
NaCl Control	—	++++	++++	—	++++	++++	—	++++	++++	—	++++	++++	—	++++	+++

TABLE II.
Titration of residual agglutinins after absorption of cow serum with cadaver and mummy tissues.

	Serum Dilutions														
	1:2			1:4			1:8			1:16			1:32		
	Cells			Cells			Cells			Cells			Cells		
Tissue Extract	O	A	B	O	A	B	O	A	B	O	A	B	O	A	B
I M 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
I M 2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
I M 3	+++	+	—	+	—	—	—	—	—	—	—	—	—	—	—
I M 4	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
I M 5	++	+	—	±	—	—	—	—	—	—	—	—	—	—	—
I M 6	++	+	—	—	—	—	—	—	—	—	—	—	—	—	—
I M 7	++	+	—	—	—	—	—	—	—	—	—	—	—	—	—
I M 8	+	+	—	±	—	—	—	—	—	—	—	—	—	—	—
I M 9	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
I M 10	++	+	—	—	—	—	—	—	—	—	—	—	—	—	—
I M 11	++	+	—	+	—	—	—	—	—	—	—	—	—	—	—
I M 12	++	+	—	±	—	—	—	—	—	—	—	—	—	—	—
Cadaver Group O	++	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Cadaver Group A	++++	+	—	++++	—	—	+++	—	—	++	—	—	—	—	—
NaCl Control	++++	+	—	++++	—	—	++++	—	—	+++	—	—	+	—	—

+, ++, +++, and ++++ = varying degrees of agglutination.
— = no agglutination.

0.5 cc. of increasing dilutions of $O(\alpha\beta)$ serum, the mixtures were allowed to remain over-night in the refrigerator and were tested the following day for the residual agglutinins with O, A, and B cells as above.

The results of this procedure were quite satisfactory. Group O cadaver tissue absorbed neither α nor β from the $O(\alpha\beta)$ serum, whereas group A tissues absorbed α agglutinin. We were unable to obtain group B cadaver tissue and therefore this was not tried.

This procedure was then applied to the study of the mummy tissues. The results given in Table I indicate that neither α nor β agglutinins were absorbed with mummy extracts nor by the group O cadaver tissue extract.

The question still remained, however, whether the mummy tissue left the α and β agglutinins intact in $O(\alpha\beta)$ serum by virtue of the absence of A and B agglutinogen in the tissue (if tissues belonged to group O), or because the isoagglutinogens, if present in the tissue, had not been extracted.

To answer this question use was made of the known property[3, 4, 5] of cow serum, previously absorbed with A_1B human cells, to agglutinate specifically human blood cells of Group O. As the results in Table II show, the specific anti-O agglutinin of such serum was absorbed by the group O cadaver tissue extract and by the mummy tissue extracts examined, whereas there was no such absorption by the group A cadaver tissue extract.

While the findings here presented are insufficient to permit one to draw a conclusion as to the original distribution of the blood groups among the American Indians, this requiring a much larger series of specimens from different sources, the findings are recorded nevertheless because they offer a procedure which may be of value in further study of similar material from other localities.

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[3] Schiff, F., *Klin. Wochenschr.*, 1927, **6.1**, 303.

[4] Greenfield, G., *Z. f. Immunitatsf.*, 1928, **56**, 107.

[5] Sasaki, H., *Z. f. Immunitatsf.*, 1932, **77**, 101.

7395 C

Composition of the Mixed Duodenal Secretions.

CHARLES M. WILHELMJ, LEO C. HENRICH AND FREDERICK C. HILL.

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In a previous communication[1] the results of studies on the composition of mixed duodenal secretions were reported. These previous studies were performed on healed duodenal pouches completely isolated from the stomach and every effort was made to avoid all complications which might alter the composition of the mixed duodenal secretions. However, it appeared necessary to confirm these results by different methods which would be free of certain hypothetical sources of error, based primarily on the operative procedures previously employed. A large number of analyses of the contents of the dogs' stomach obtained by lavage 24 hours after a light feeding of milk and syrup have shown that under these conditions hydrochloric acid is either absent or present in very small amounts. It therefore seemed that duodenal contents obtained from the intact duodenum, 24 hours after a light feeding of milk and syrup, would be practically free from contamination by acid gastric contents. Gastrostomy openings were made low on the body of the stomach through which a duodenal tube was inserted and the tip allowed to pass into the duodenum. When the dogs were lying on the side, the main cavity of the stomach was usually below the opening into the duodenum. At frequent intervals during the experiments a small rubber catheter was passed into the stomach and an attempt made to aspirate gastric secretions. Nothing but a small amount of tenacious mucus was ever obtained. The duodenum was stimulated by introducing a standard hydrochloric acid solution (approximately one-tenth normal) containing phenol red through the duodenal tube. After a latent period of several minutes' duration a copious flow of duodenal secretions occurred. The secretions aspirated from the duodenum often contained some of the acid-phenol red solution which had been introduced but the phenol red was too dilute to determine accurately, hence the duodenal secretions were added to approximately 25 cc. of the same strength acid-phenol red solution that had been introduced into the duodenum. The samples were always bile-stained but occasionally a large amount of dark, viscid bile was suddenly added, due apparently to emptying of the gall bladder. Samples containing large amounts of gall bladder bile were usually discarded because of the difficulty in removing the bile before determining the percent of phenol red.

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[1] Wilhelmj, C. M., Neigus, I., and Hill, F. C., *Am. J. Phys.*, 1934, **107**, 490.

The following analyses were performed on the acid-phenol red solution containing the duodenal secretions: (1) The percent of phenol red after treating with two-thirds normal sulfuric acid and 10% sodium tungstate to remove mucus and bile pigments. (2) Total and mineral chloride after ashing of the dried samples.

Determination of the percent of phenol red present showed the amount of duodenal secretions that had been added to the acid-phenol red solution. The chloride concentration of the original acid-phenol red solution, when multiplied by the percent of phenol red in the mixture, corrected the original acid solution for dilution. The amount of acid chloride present after the addition of duodenal secretion was always below the corrected value due to neutralization of acid by the duodenal secretions. The total chloride present after the addition of duodenal secretions was always above the corrected value and this extra chloride was the chloride present in the duodenal secretions. Further details have been given[2] and need not be repeated here.

The results of 21 experiments performed on 3 dogs are given in detail in Table I. Two points deserve special emphasis.

1. *Chloride Concentration of the Mixed Duodenal Secretions.* The average value for 21 experiments was 307 mg. per 100 cc. and the individual values ranged from 210 to 377 mg. In the previously reported studies on isolated duodenal pouches, 19 experiments on 3 animals gave an average value of 310 mg. per 100 cc. with individual variations of approximately the same magnitude.

2. *The Alkalinity of the Mixed Duodenal Secretions.* The average value for 21 experiments was .04 normal with individual values ranging from .01 to .08 normal. In the previous studies on isolated duodenal pouches, 19 experiments averaged .04 normal with approximately the same range of variation.

The low average alkalinity of the mixed duodenal secretions is a rather surprising finding but becomes clear when it is remembered that of the 3 secretions comprising the mixed duodenal secretions (bile, *Succus entericus* and pancreatic juice) pancreatic juice is the only one which contributes definite alkalinity. The alkalinity of pure pancreatic juice according to Gamble and McIver,[3] Hartman

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[2] Wilhelmj, C. M., Neigus, I., and Hill, F. C., *Am. J. Phys.*, 1933, **106**, 381.

[3] Gamble, J. L., and McIver, *J. Exp. Med.*, 1928, **48**, 837, 849.

TABLE I.

Dog	Acid Solution Used mg. Cl per 100 cc.	Total Cl mg. per 100 cc.	Mineral Cl mg. per 100 cc.	Total Minus Mineral Cl (Acid Cl)	PSP %	Cl Original Acid Solution (Corrected for Dilution)	Extra Cl mg. per 100 cc.	Cl Acid Deficit mg. per 100 cc.	Cl Concentration Duodenal Secretion mg. per 100 cc.	Cl Acid Deficit / cc. of Duodenal Secretion	Normality of Duodenal Secretions
1	350	348	103	245	81	284	64	39	336	2.1	.06
		358	89	269	79	277	81	8	386	0.4	.01
		355	93	262	82	287	68	25	377	1.4	.04
		351	75	276	85	298	53	22	353	1.5	.04
1	348	330	201	129	63	219	111	90	300	2.4	.07
		324	188	136	61	212	112	76	288	2.0	.06
		328	195	133	63	219	109	86	295	2.3	.06
2	347	342	96	246	82	284	58	38	322	2.1	.06
		331	100	231	82	284	47	53	261	2.9	.08
		336	47	289	88	306	30	17	250	1.4	.04
		342	84	258	82	284	58	26	322	1.4	.04
2	347	330	125	205	70	243	87	38	290	1.3	.04
		348	125	223	71	246	102	23	352	0.8	.02
		339	113	226	77	267	72	41	313	1.8	.05
3	352	339	54	285	82	289	50	4	278	0.2	.01
		342	50	292	86	303	39	11	278	0.8	.02
		336	44	292	89	313	23	21	210	1.9	.05
		336	46	290	86	303	33	13	236	0.9	.03
3	348	342	95	247	76	264	78	17	325	0.7	.02
		345	89	256	79	275	70	19	334	0.9	.03
		346	80	266	79	275	71	9	338	0.4	.01
Average									307	1.4	.04

Complete data in 21 experiments in which duodenal secretions were added to approximately tenth normal hydrochloric acid solutions containing phenol red.

and Elman,[4] Elman and McCaughan,[5] and Starling[6] ranges from 0.06 to 0.1 normal. Since the relative proportion of the 3 separate secretions comprising the mixed duodenal secretions may vary in individual samples, the range of alkalinity may theoretically vary between that of *Succus entericus* and bile to a value somewhat lower than that of pancreatic juice. The average alkalinity of .04 normal indicates that in the mixed duodenal secretions the pancreatic juice is diluted by varying amounts of the nearly neutral bile and *Succus entericus*, the average dilution being approximately 50%. The average low alkalinity of the duodenal secretions makes it obvious that the effect in lowering the acidity of tenth normal hydrochloric acid depends more upon dilution than upon neutrali-

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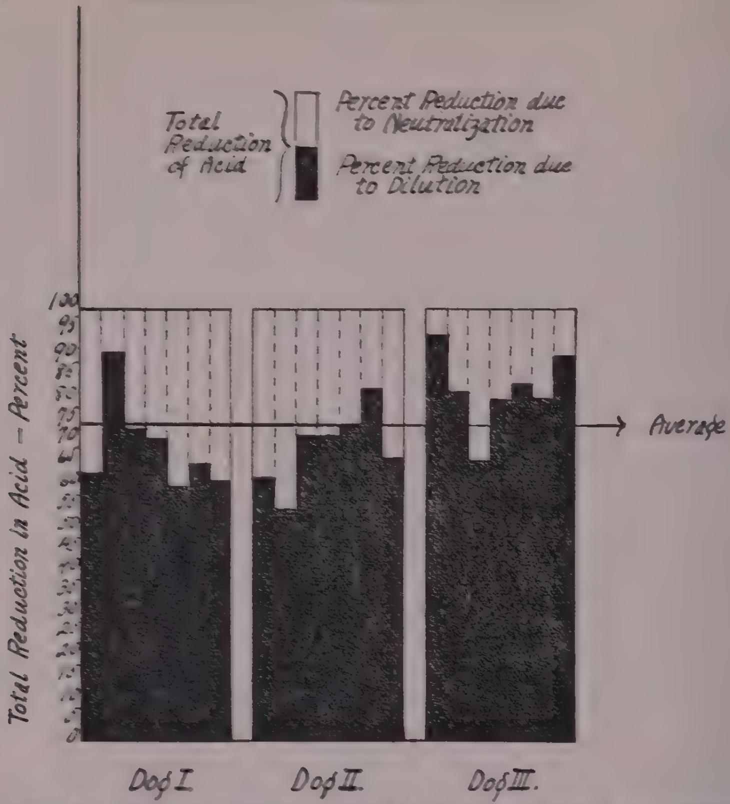
[4] Hartman, A. F., and Elman, R., *J. Exp. Med.*, 1929, **50**, 387.

[5] Elman, R., and McCaughan, J. M., *J. Exp. Med.*, 1927, **45**, 561.

[6] Starling, E. H., *Principles of Human Physiology*, Lea and Febiger, 1926.



Shows the relative importance of dilution and neutralization in the reduction of the acidity of approximately tenth normal hydrochloric acid by duodenal secretions.

zation: (Fig. 1), on the average, 73% of the reduction in acidity is due to the diluting effect and only 27% to neutralization.

The chloride concentration of mixed duodenal secretions is definitely lower than that of the secretion of the fundic portion of the stomach. When duodenal secretions regurgitate into the stomach the chloride concentration of the fundic secretion will be lowered by dilution with the duodenal secretions.

Since the chloride present in mixed duodenal secretions is neutral chloride it is obvious that when bile is present in gastric contents, the entire neutral chloride content cannot be considered as arising from neutralization of hydrochloric acid. When duodenal secretions are mixed with approximately tenth normal hydrochloric acid, only about 1.5 mg. of neutral chloride out of every 5 mg. actually represent neutralized hydrochloric acid.

Summary. The average alkalinity of the duodenal secretions was found to be approximately .04 normal and the chloride concentration 307 mg. per 100 cc. These values obtained on secretions from the intact duodenum are in excellent agreement with values previously reported on secretion obtained from isolated duodenal pouches.

7396 P

Methylene Blue as an Agent for Reducing Red Blood Cell Count.

WILLIAM B. WENDEL AND MARTHA LOU HEFLEY. (Introduced by W. R. Amberson.)

From the Department of Chemistry, University of Tennessee, College of Medicine, Memphis.

Over a period of a week or 10 days following intravenous injection of methylene blue into dogs poisoned with cyanide there occurs a decrease in the oxygen capacity of the blood and a corresponding decrease in red blood cell count. The same effect is observed when the dye alone is given. Results of a typical experiment are reported in this preliminary note.

Table I shows the changes in red blood cell count, oxyhemoglobin (by O_2 capacity method), and reticulocytes in a 17 kilogram dog

TABLE I.

Date	Time A.M.		Oxyhemoglobin mMols. per liter	R.B.C. in millions	Reticulocytes %
3-6-34	11:30	Blood No. 1	9.18		
	11:31	26 cc. 1% methylene blue injected			
	P.M.				
	3:20	Blood No. 2	(Methylene blue and methemoglobin not detected spectroscopically)		
	3:25	23 cc. 1% methylene blue injected			
3-10-34		Blood No. 3	3.76		
3-12-34		” ” 4	2.23	1.56	1.4
3-13-34		” ” 5	2.18	1.81	
3-14-34		” ” 6		1.62	7.4
3-15-34		” ” 7*	(White cell count only)		
3-16-34		” ” 8	3.26	2.67	4.6
3-19-34		” ” 9	4.63	3.36	5.2
3-21-34		” ” 10		3.07	3.4
3-27-34		” ” 11		3.82	2.0
3-29-34		” ” 12†	6.60	4.23	2.0

* Whites, 10,200. † Whites, 13,600.

following intravenous injection of 30 mg. of methylene blue per kilo body weight.

Each of 5 dogs which received methylene blue (in quantities ranging from 15 to 40 mg. per kilo body weight) developed an anemia the severity of which was greatest with the larger doses. In 2 experiments, otherwise less complete than that reported, the oxyhemoglobin (O_2 capacity) of the blood was determined on the first and second days following methylene blue injection and was found to be only slightly below the preinjection level, although several days later it had fallen to severely anemic levels. This fact and the absence of hemoglobinuria and hematinuria during the entire period following dye injection indicate that there is no immediate massive destruction of cells in the circulation.

Experiments designed to relate quantitatively dosage to response and to determine the mechanism of the methylene blue action are in progress. We also have in mind the possibility that methylene blue may find practical application in the treatment of polycythemia vera.

7397 P

Conduction of Cortical Impulses to the Autonomic System.

W. C. HUNSICKER, JR., AND E. A. SPIEGEL.

From the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia.

Clinical as well as experimental observations show that the cortical centers of vegetative organs are, in many instances, in close topical relation to those of adjacent skeletal muscles. The descending cortico-fugal tracts to the peripheral part of the autonomic system are topographically related to the pyramidal tract. They also lose their excitability after destruction of the motor cortex and frontal lobe, within the same time as do the corticofugal somatic fibers. (Spiegel,[1] Friedberg.[2]) It seemed, therefore, of interest to ascertain whether the pyramidal tracts by themselves can conduct corticofugal impulses to the autonomic system. The experiments were performed on 30 cats. In a first series, the "extrapyramidal" fibers that arise from the hypothalamus were severed by deep incision into the floor of the 4th ventricle. The central grey matter and tegmentum in the pons and midbrain were destroyed where the centrifugal fibers of the hypothalamus are located. In a second series of experiments, the conduction of cortical impulses to vegetative organs was studied after transverse section of both pyramidal tracts on the base of the pons. After the lesion of either of these systems had been performed, the effects of stimulation of the motor cortex and the frontal lobe was noticed on the pupil, the blood vessels, the heart, the sweat glands and the urinary bladder.

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[1] Spiegel, E. A., *Zentren. des autonomen Nervensystems.* Berlin, Springer, 1928. *Bull. Johns Hopkins Hosp.*, 1932, **50**, 237.

[2] Friedberg, Ch., *Z. ges. Neurol.*, 1931, **50**, 134.

In the study of the *pupil,* the lesion of the brain stem was combined with unilateral cervical sympathectomy. Thus, cortical stimulation elicited on the pupil ipsi-lateral to the sympathectomy only inhibition of the sphincter, on the other pupil inhibition of the oculomotor plus stimulation of the sympathetic.

Severance of the systems arising from the hypothalamus produced a myosis and prolapse of the nicitating membrane that in some cats was not at all, and in others but slightly changed after cervical sympathectomy. Section of the pyramidal tracts was not followed by myosis. In stimulation of the cortex the dilatation of the pupil with intact cervical sympathetic was more pronounced when the pyramidal tracts were severed than when the hypothalamic pathways were cut. At times maximal dilatation of the pupil was observed in cortical stimulation despite pyramidal destruction. In other words, the pyramidal tract has no such tonic influence upon the spinal segmental centers of the cervical sympathetic as has the hypothalamus, the pupillodilatator centrifugal cortical impulses use the hypothalamic systems to a larger extent than the cortico-spinal neurones.

The *blood pressure* was recorded before as well as after curare paralysis was produced. After curarization vaso-constrictor reactions were more rarely observed in cortical stimulation than vasodilator reactions. These reactions were present in both series of experiments, but were somewhat better preserved when only the pyramidal tracts were severed than after section of the fibers dorsally to the peduncles.

In some experiments, the *electrocardiogram* was recorded. Only a relatively small change of the heart-rate (slight tachycardia as well as slight bradycardia or both reactions following each other) were brought about. Such changes were observed in stimulation of the frontal lobe with the brain stem intact as well as after both types of lesions.

A similar result was obtained in studying the activity of the *sweat glands* under cortical influence by recording the galvanic skin response. The reaction observed after both types of lesions of the brain stem was as a rule weaker than with the brain stem intact, particularly after severance of the hypothalamic tracts.

On the *bladder,* cortical stimulation produced stimulating as well as inhibitory effects. It is interesting to note that not only the stimulating, but also the inhibitory reactions depend mainly upon the pelvic nerves. The increase of bladder activity as well as the inhibition of this organ were observed in both series of experiments. The severance of the extra-pyramidal tracts did not impair these reactions to a greater extent than did the section of the pyramidal tracts.

It is concluded that there exists a double (pyramidal and extra-pyramidal) conduction of cortico-fugal impulses to the autonomic centers in the cord.

7398 P

Mechanism of Invasiveness in the Genus Streptococcus.

E. W. DENNIS AND D. A. BERBERIAN. (Introduced by G. H. Miller.)

From the Department of Bacteriology and Parasitology, American University of Beirut.

Menkin[1] recently demonstrated that various materials are fixed *in situ* when injected into an acutely inflamed area, and that the mechanism of fixation of such substances as bacteria and trypan blue is primarily mechanical obstruction in the form of a fibrinous net-work and thrombosed lymphatics at the site of inflammation. Obviously the speed with which an inflammatory irritant causes the region to be blocked off is a significant factor in determining its ability to disseminate into the contiguous tissues or blood stream. Menkin[1] observed that *Staphylococcus aureus* caused the formation of thrombi in the lymphatics and fixation of trypan blue within about one hour after their introduction into the skin of rabbits, while the more invasive *Streptococcus hemolyticus* produced fixation only about 45 hours later. Menkin believed that the delayed fixation in the latter case was due to the "relatively mild local effects" of the streptococcus.

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[1] Menkin, V., *J. Exp. Med.,* 1933, **57**, 977.

Using Menkin's[1] method, we have fully confirmed his observations of the behavior of *Strep. hemolyticus* and *Staph. aureus in vivo*, but *our additional data show that the delayed inflammatory fixation of streptococci is due to the production by these organisms of a substance or substances, active both in vivo and in vitro, which when present in sufficient concentration is capable of inhibiting inflammatory fixation for many hours, thus enabling the organisms to disseminate relatively freely through the lymphatics.*

Our *in vivo* observations have been carried out on more than 150 rabbits. The organisms studied were the Dochez and Griffith scarlatinal strains and Birkhaug's E_1 erysipelas strain of *Strep. hemolyticus*, a virulent strain of *Strep. viridans*, and 2 strains of *Staph. aureus*. The "fixation time" for each strain was: Dochez 48-56 hr.; Griffith 48 hr.; E_1 48 hr.; *Strep. viridans* 36 hr.; *Staph. aureus* 1-2 hr. The staphylococcus strains used always produced fixation of the dye in less than 2 hours.

The inhibitive properties of streptococcus cultures and filtrates were demonstrated by injecting 0.5 cc. amounts into the skin of the fore legs of a series of rabbits, and 20 minutes later introducing a suspension of 5 billion *Staph. aureus* (0.5 cc.) into the same area. Injection of 0.4 cc. of trypan blue into the inflamed areas at appropriate intervals and subsequent examination of the axillary lymph nodes for the presence of the dye, showed that the filtrates and cultures introduced were capable of delaying the fixation of the dye for at least 4 hours, despite the antagonistic action of the staphylococcus. All of the invasive strains of *Streptococcus* studied have shown inhibitive properties, and there is some evidence that the more invasive strains of *Staph. aureus* may delay fixation.

Rabbits immunized against *Strep. hemolyticus* showed a reduction of the fixation time from 48 hr. to 6 hr.

Our *in vitro* studies indicate that the mechanism by means of which inflammatory fixation is inhibited may be different for different kinds of streptococci. The action of the different strains on human and rabbit plasma clot has been tested according to the method of Tillett and Garner,[2] with the following results:

Strain	*Human plasma clot*	*Rabbit plasma clot*
Dochez	Rapid lysis	Rapid clot retraction
Griffith	Rapid lysis	Rapid clot retraction
E_1	Inhibition of clot formation	Inhibition of clot formation
Strep. viridans	Inhibition of clot formation	Inhibition of clot formation

[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

The lytic property of the Dochez and Griffith filtrates is relatively resistant to heat, one hour at 100°C. in the water bath being required to destroy it, although it is noticeably weakened after 40 minutes. The factor which inhibits clot formation is even more thermostable, since boiling for 1 hour seldom shows any weakening effects.

7399

Experimental Angina Pectoris in the Dog. Effect of Total Thyroidectomy.

PHILIP SHAMBAUGH.* (Introduced by E. C. Cutler.)

From the Laboratory of Surgical Research, Harvard Medical School.

The clinical observation that the beneficial effect of total thyroidectomy in angina pectoris is an almost immediate one, and so cannot be due entirely to the fall in the basal metabolic rate which does not begin to occur for over a week, has given rise to speculation as to the possible mechanism involved.

We investigated the possibility that this effect might be due to interruption of nerve impulses from the heart. Our method was that described by Sutton and Lueth[1] for producing "experimental angina pectoris" in the dog consisting in placing a ligature about the anterior descending branch of the left coronary artery and compressing the coronary vessels by traction on this ligature. This method was successfully employed by White and his associates[2] to test the efficacy of various surgical procedures in interrupting the afferent nervous pathways of the heart. Five dogs were tested in this manner after removal of both thyroids. One animal was tested 5 hours after thyroidectomy, and 4 dogs on the eleventh, thirteenth, fourteenth, and twenty-eighth day respectively. A definite discomfort response was elicited in each instance. Two of the dogs had been similarly tested previous to the thyroidectomy and in these no diminution was noted in the amount of traction required to produce the typical response.

The relief of angina by removal of the thyroid may be due to the diminished sensitiveness to adrenalin[3] which has been demonstrated clinically as early as 24 hours following the operation. This implies that the individual attacks of angina pectoris in a patient suffering from this disorder may be precipitated by a sudden increase in the patient's physiological output of adrenalin. We attempted to precipitate the discomfort response in dogs by injecting adrenalin. This was successfully accomplished in 9 dogs by applying a constant *subminimal traction* on the coronary suture by means of weights suspended over pulleys, and then raising the blood pressure suddenly by injecting adrenalin into the femoral vein. The amount of traction which was maintained without discomfort varied from 50 to 800 gm. and the dosage of adrenalin, which in the presence of this subminimal traction elicited the typical discomfort response, varied from 0.5 cc. of 1:10,000 to 0.2 cc. of 1:1,000 solution. In each case the injection of the same amount of adrenalin without the subminimal traction did not cause the slightest evidence of discomfort.

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* Arthur Tracy Cabot Fellow.

[1] Sutton, D. C., and Lueth, H. C., *Arch. Int. Med.*, 1930, **45**, 827.

[2] White, J. C., Garrey, W. E., and Atkins, J. A., *Arch. Surg.*, 1933, **26**, 765.

[3] Eppinger, E. C., and Levine, S. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 485.

We believe that the production of discomfort in the dog by the above method probably simulates the attack of angina pectoris in humans more closely than does the older method in which direct traction alone is used.

It has been found possible to elicit the typical response by this indirect method after thyroidectomy as well as before. However, the nature of the procedure makes it impossible to repeat the exact experimental conditions a second time, so it is not possible to test accurately the effectiveness of the same dose of adrenalin following removal of the thyroid. We have studied the vaso-pressor effect of a given dosage of adrenalin before and after thyroidectomy in dogs, and although it has been markedly diminished in certain animals, this has not been a constant finding, perhaps due to the frequent occurrence of accessory thyroid tissue in the dog.

7400 P

Local Cerebral Anaphylaxis in the Dog. II.

LEO M. DAVIDOFF AND NICHOLAS KOPELOFF.

From the Department of Bacteriology, Psychiatric Institute, New York City.

Experimental data were presented[1] on 5 dogs in which each was sensitized with horse serum in the left cerebral motor area after injury to this region. Following an interval of 2 to 4 weeks they received intravenous injections of horse serum. These injections were followed in 4 of the 5 animals by transient right-sided hemiparesis.

The present communication concerns similar experiments on 35 dogs. Three, serving as controls and receiving normal saline into the brain instead of sensitizing protein, showed no subsequent focal reaction on repeated intravenous injection of the test protein. Twenty-one of the remaining 32 dogs showed evidence of local cerebral irritability after intravenous injection of the same protein with which the brain had been previously sensitized. In many of the dogs the local reaction could be repeated and *was always confined to the side opposite the sensitized cerebral hemisphere.* In a few animals which were kept alive over a long period of time the same reaction occurred almost as often as the intravenous injection was made, provided proper intervals between injections were allowed. One animal showed a focal convulsive seizure which became generalized and eventuated in death.

For the most part horse serum and egg albumen were used as the sensitizing proteins. A few protocols are cited to bring out special points:

DOG No. 17. Small female Airedale.

1- 7-32. The left side of the brain was exposed and an agar disc containing horse serum, 1 mm. thick and 1.5 cm. in diameter, was placed against the exposed area and held in place by suturing the temporal muscle over it.

1-12-32. 1 cc. of horse serum was injected intravenously. In a few minutes symptoms of general anaphylaxis appeared (defecation and urination).

2-26-32. 2 cc. of horse serum were injected intravenously. In 2 minutes defecation and urination occurred. In 12 minutes *slight but definite dragging of the right forepaw was observed.*

3- 9-32. Into a pocket formed by a small rubber ring placed over the *right* cerebrum 0.1 cc. of fresh egg albumen was injected between the dura and pia mater.

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[1] Davidoff, L. M., and Kopeloff, N., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 71.

4- 1-32. 5 cc. of fresh egg albumen were injected intravenously. In 2 to 4 minutes the dog defecated and urinated. *Definite moderately severe weakness of the left forepaw was noted.*

This experiment is particularly valuable in that each antigen served as a control on the other. It is evident that the focal weakness observed was no mere coincidence but occurred as a result of a reaction which had taken place in a previously sensitized (even though uninjured) area of brain tissue, following the injection of the same antigen into the blood stream.

Another dog whose protocol is omitted was sensitized to horse serum placed in a collodion sac between the pia and the dura mater on the right side of the brain. When injected with horse serum intravenously 3 weeks later this dog developed weakness in the left extremities followed by twitching of the left side of the face (Jacksonian seizure). Generalized convulsions, coma and death followed within one hour.

DOG No. 40. Irish tan and white female terrier.

3- 9-33. This dog which had previously received a number of injections of horse serum both into the left cerebrum through a trephined hole and later intravenously, without signs of anaphylaxis, was now injected with 1 cc. of fresh egg albumen into the left brain by way of the defect in the skull. No reaction occurred.

3-21-33. 1 cc. of fresh egg albumen was injected into the left cerebrum.

1-22-34. 0.5 cc. of fresh egg albumen was injected into the left cerebrum.

2-13-34. 4.5 cc. of fresh egg albumen were injected intravenously. Within 2 minutes vomiting occurred. Within 3 minutes *definite weakness of the right foreleg* lasting several minutes was observed.

2-15-34. 1 cc. of fresh egg albumen was injected intravenously. No reaction followed.

2-16-34. 5 cc. of egg albumen were injected intravenously. Almost immediately there was slight vomiting. Within 4 minutes local flushing occurred on the *left* side of the head, later spreading to the face, ear, conjunctiva, and eventually, but less markedly, to the right side of the head, face, etc. Within 10 minutes there were clonic spasmodic contractions of the muscles of the right shoulder girdle and foreleg with increasing weakness of this extremity. This weakness lasted about 24 hours.

2-19-34. 5 cc. of egg albumen were injected intravenously. The results were essentially like those observed on 2-16-34.

2-21-34. 5 cc. of egg albumen were injected intravenously. The same reaction was noted as on 2-16-34 but was of lesser intensity.

3-23-34. 5 cc. of egg albumen were injected intravenously. The skin reaction and clonic jerkings as well as weakness of the right anterior extremity again occurred in a pronounced manner.

This dog is still alive and is to receive monthly intravenous injections of egg albumen. The experiment outlined above with this animal illustrates several points: 1. Repeated intravenous injections of the same antigen did not appear to desensitize the animals. 2. Repeated injections at short intervals reduced the degree of severity of the reaction while increasing the interval tended to restore the vigor of the response. 3. The skin over the scalp which was apparently accidentally sensitized during the injection into the brain acted as a graphic indicator of what was taking place in the cerebrum following the intravenous injection.

7401 C

Effect of Splenectomy on Natural Resistance of Albino Rats and Mice to Histamine Poisoning.

DAVID PERLA.

From Division of Laboratories, Montefiore Hospital, New York.

In order to determine whether the drop in natural resistance following the removal of the spleen is of a general character or is specific for certain types of infections and toxemias, the effect of the removal of the spleen in rats and mice on their resistance to histamine was observed. In these studies rats free of *Bartonella muris* infection and mice free of *Bartonella muris, Eperythrozoon coccoides* and *Klossiella muris* were used.[1] These latent infections are adversely influenced by splenectomy.[1]

Ten splenectomized rats of Wistar stock received ergotamine acid phosphate one week after operation in amounts ranging from 700 to 1200 mg. per kg. of body weight. Those receiving 1000 to 1200 mg. per kg. died; the rest survived. Of 10 control rats the M.L.D. was found to lie between 1000 and 1200 mg. per kg. of body weight.

A similar number of splenectomized mice free of latent infections likewise survived as large an amount of ergotamine acid phosphate as the control mice. The M.L.D. of histamine in the mice of this strain was 1700 to 2000 mg. per kg. of body weight.

Splenectomy in rats and mice free of latent infections does not alter the resistance of these animals to histamine poisoning. In view of the fact that the removal of the spleen depresses the natural resistance to many infections, it is interesting that the resistance to a chemical poison such as histamine is unaffected.

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[1] Marmorston, Jessie, in press.

Bilateral suprarenalectomy, on the other hand, lowers the natural resistance of rats to all toxemias, infections and poisons. In contrast to suprarenalectomy, splenectomy specifically affects the resistance to certain types of infections and toxemias, but the drop in natural resistance is not of a general character.[2]

7402 C

Compensatory Changes Following Splenectomy in Bartonella Free Rats.

DAVID PERLA.

From Division of Laboratories, Montefiore Hospital, New York.

In rats that were carriers of *Bartonella muris*, certain changes were observed, following splenectomy and recovery from the anemia, in the hemocytoblastic tissue and in the reticular and endothelial elements. These changes appeared 3 to 5 months after splenectomy and were associated with immunity to further infection with *Bartonella muris*. The changes consisted primarily of hyperplasia of hemolymph tissue, hyperplasia of reticular and endothelial elements of lymph nodes, the formation of lymphoblastic foci periportally in the liver and peribronchially and perivascularly in the lung, regeneration of all elements of the thymus and marked hyperplasia of all elements of bone marrow (increased hematopoiesis).[1]

In view of the presence of coincident latent infection in the rat, the compensatory changes described following splenectomy in rats of Bartonella carrier stock were difficult to evaluate. What was due to the absence of the spleen and what to previous *Bartonella muris* anemia remained uncertain. Studies were therefore made in rats in which the exacerbations of latent infections following splenectomy could be excluded. Six Bartonella-free rats, 3 months of age, of Wistar stock, were splenectomized and killed 6 months later. Six unsplenectomized rats of the same age and stock were killed at the same time. The presence of Bartonella infection was excluded by red blood cell counts and smears of peripheral blood at frequent intervals.

The anatomical changes observed were similar to those found in splenectomized Bartonella-carrier rats but less extensive. The liver showed little if any perivascular lymphocytic infiltration. The Kupffer cells were not enlarged, nor were they more prominent in phagocytic activity than in normal rats. The hemolymph nodes in the retroperitoneal area and the mediastinum were enlarged and showed endothelial cell proliferation with marked phagocytosis of red cells and blood pigment. Hemosiderin pigment was also present in the sinus endothelial cells and reticular elements of lymph nodes and proliferation of these cells occurred. Peribronchial accumulation of lymphocytes and pale reticular elements were present in 4 of the splenectomized rats but absent in 2. The thymus was rich in cellular elements and resembled the thymus of the immature rat, but it was not hypertrophied. The bone marrow was hyperplastic.

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[2] Perla, David, and Marmorston, Jessie, *Arch. Path.*, 1933, **16**, 379.

[1] Marmorston-Gottesman, J., and Perla, David, *J. Exp. Med.*, 1931, **53**, 877.

It may be that the difference in degree of stimulation of the hemocytoblastic tissue and of the cells of the macrophage system is greater in Bartonella-carrier rats than in Bartonella-free rats, due to the greater physiological need created by the presence of latent infection.

Summary. The late compensatory changes following splenectomy in Bartonella-free rats are essentially similar to, but less extensive than those observed in Bartonella-carried stock.

7403 C

The Habitat of Trichophyton Interdigitale Outside the Body.

JOHN W. WILLIAMS.

From the Homberg Memorial Infirmary and the Department of Biology and Public Health, Massachusetts Institute of Technology.

Adequate scientific proof of the existence of *Trichophyton interdigitale* (organism of "Athlete's Foot") outside the body seems lacking. Statements in the literature, however, would lead one to judge otherwise. The U. S. Public Health Service states[1]: "Bath mats are justly blamed and it is probable that ringworm can be acquired from them just as warts on the soles of the feet can be . . . . . it can also originate in hotels and from the use of infected towels or soap." The Public Health Engineering Section of the American Public Health Association[2] states: "Most if not all of these foot diseases are caused by a fungus which is spread by infection of the floors of dressing rooms, etc., at pools, bath houses, gymnasiums, and other places where persons go barefoot." While these inferences may be true they are unaccompanied by cultural proof.

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[1] *Health News,* E-28, U. S. Public Health Service. September 18, 1928.

[2] *Am. J. Public Health,* 1930, **20**, 7.

Bonar and Dryer[3] made 30-40 plants of scrapings of floors, hair, etc., in cracks in the floor and traps on the drains from showers. They were troubled by excessive overgrowth with other organisms. They failed to recover the *Trichophyton interdigitale*.

Because of scarcity of cultural work we felt such a study of showers, boat houses and gymnasia would be valuable.

Cotton applicators were moistened with normal saline, placed in test tubes which were plugged with cotton and autoclaved. The applicators were used to mop the area to be studied. They were then cut near the cotton end with a flamed scissors and this portion allowed to drop into a sterile petri dish. Melted Sabouraud's proof medium was poured over the swab and the medium agitated by sliding the dish back and forth on the table. In this manner an even distribution of colonies was obtained. The petri dishes were observed over a period of a month and transplants made on Sabouraud's proof slants of colonies which appeared the least suspicious.

Approximately 1500 cultures were made, one-half of which were of student lockers. Razor blades which were flamed each time were used in making the scrapings. Floors, machines, shells, benches, sills, mats, gymnasia apparatus, showers, etc., were cultured. Several cultures were made of sock washings, beach sand, beard hair and scalp hair.

Two suspicious colonies were obtained, one from a locker and one from a machine foot rest. We failed, however, to obtain sufficient differentiation to prove to our complete satisfaction that the organisms were either *Trichophyton interdigitale* or *Epidermophyton inguinale*. Many white mycelial colonies which might well have been "pleomorphic" forms of these organisms were noted. It is possible that this is the form in which the organism occurs in nature and from which it becomes infectious by a process not yet understood. Proof of this rests entirely on additional knowledge gained in the subject.

The fact that this school apparently has few cases of "Athlete's Foot" may account for our inability to find a typical example of the organism. In the school year of 1931-32 seventeen cases reported for treatment at the clinic and in the year 1932-33 only 14 cases, out of a student population of approximately 3,000. We have been unable to trace any infection to a focus about the Institute. The fact that the students diligently use foot baths and care for their feet so well may be a factor in the comparative rarity of the disease.

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[3] Bonar, Lee, and Dreyer, Alice Donsler, *Am. J. Public Health*, 1932, **22**, 909. Bonar, Lee, personal communication, Sept. 19, 1933.

7404 C

Effect of Glycerine on the Growth of Certain Pathogenic Fungi.

JOHN W. WILLIAMS.

From the Department of Biology and Public Health, and the Homberg Memorial Infirmary, Massachusetts Institute of Technology.

In studying a group of fungi the inhibitory effects of varying concentrations of glycerine were tabulated from the point of view of possible differential features. Glycerine was incorporated in Sabouraud's proof medium and the pH adjusted to 5.5. Concentrations of 1, 5, 10, 15, 20 and 25% were used.

Growth of the following pathogenic fungi and the 2 saprophytes, *Lichtheimia sp.* and *Scopulariopsis brevicaulis,* was observed over a period of 30 days: *Achorion schoenleinii, Acladium castellani, Candi-*

TABLE I.
Glycerine in Sabouraud's Proof Media.

	0%	1%	5%	10%	15%	20%	25%
Achorion schoenleinii	3	5	5	5	7d	7d	21s
Acladium castellani	3	5	5	5	5	5d	5d
Candida candida	3	3	3	3	3	3	3
Endodermophyton tropicale	5	5	5	7	7d	7d	—
Endomyces capsulatus	5	5s	5s	7s	7s	—	—
Endomyces dermatitidus	5	5	5s	7s	7s	—	—
Epidermophyton inguinale	3	3	3	3	7s	14s	—
Glenospora gammeli	5	7s	7s	7s	14s	—	—
Geotrichum bachmann	3	3	3	3	3	3	—
Indiella americana	3	5	5	5	—	—	—
Lichtheimia sp.	3	3	3	3	3	3	5
Monilia albicans	3	3	3	3	3	3	3
Microsporon apiospermum	3	3	3	7	7s	—	—
Microsporon audouini	3	3	3	3	14	30s	30s
Oöspora humi	3	3	3	3	3	3	—
Scopulariopsis brevicaulis	3	3	3	3	3	3	3
Trichophyton crateriforme	3	3	3	3	7s	7s	—
Trichophyton granulosum	3	3	3	3	5	7	—
Trichophyton interdigitale	3	3	3	3	3	5	8
Trichophyton japonicum	3	3	3	3	5	5	14s
Willia anomala	3	3	3	3	3	3	3s

da candida, Endodermophyton tropicale, Endomyces capsulatus, Endomyces dermatitidus, Epidermophyton inguinale, Glenospore gammeli, Geotrichum bachmann, Indiella americana, Monilia albicans, Microsporon apiospernum, Microsporon audouini, Oöspora humi, Trichophyton crateriforme, Trichophyton granulosum, Trichophyton japonicum, Trichophyton interdigitale, Willia anomala.

Table I gives the days within which growth was observed. The *d* indicates a marked decrease in the amount of growth, and *s* a scant growth.

Achorion schoenleinii in addition to developing a smaller colony with increase in concentration of glycerine, became more translucent in concentrations of 15% and above.

Acladium castellani gradually lost its color with increase in concentration of glycerine, becoming less granular and more waxy in appearance.

Candida candida gradually became more translucent with increase in concentration of glycerine.

Endodermophyton tropicale with increase in concentration of glycerine lost its granular white appearance and became more translucent.

Endomyces capsulatus became increasingly more translucent, its mycelia becoming subsurface.

Endomyces determatitidis. The mycelia became increasingly more sub-surface, starting to a marked degree with the 1% concentration of glycerine.

Epidermophyton inguinale. The growth became less marked and more transparent as concentration of glycerine increased.

Glenospora gammeli. There was immediate inhibition of growth with 1% glycerine, the mycelia becoming subsurface.

Geotrichum bachmann took on a more frosted appearance as concentration of glycerine increased.

Indiella americana. The colonies took on a radiating character in the 10% concentration of glycerine with a center and rim of fine white mycelia.

Lichtheimia sp. The mycelia became more sparse so that a surface sheen became apparent.

Monilia albicans became less latticed in appearance and more transparent as concentration of glycerine increased.

Microsporon apiospernum with increased concentration (5 and 10%) of glycerine developed a more pronounced black base, the mycelia finally becoming grey and losing the white appearance seen on Sabouraud's.

Microsporon audouini. Growth became coarse, granular, white and scant in 15% concentration of glycerine with mycelia largely subsurface.

Oöspora humi became more transparent, waxy and granular as concentration of glycerine increased.

Scopulariopsis brevicaulis beginning with the 5% concentration gradually lost its tan color, becoming white, finely granular, opaque, wrinkled, with a few white tufts of mycelia confined close to the surface of the media.

Trichophyton crateriforme became more wrinkled with mycelia largely subsurface.

Trichophyton granulosum. Colonies became more individual.

Trichophyton interdigitale. This organism which showed pleomorphism to a marked degree developed a red hue in the 10% concentration, became more granular and surface wrinkled in the 20 and 25% concentrations.

Trichophyton japonicum. There was a decrease in growth until in 20% glycerine only a few mycelia were observed on a frosted surface and sparse subsurface mycelia in 25% glycerine.

Willia anomala became more waxy with a decrease in concentration of glycerine.

In general, there was considerable variation in the inhibitory power of glycerine.

Grigorakis[1] made observations on the growth of *Microsporon felinum, Fox and Blaxall* (*Closterospona felineu* Grigorakis) and *Trichophyton asteroides* Sabouraud (*Spiralia asteroides* Grigorakis) on a medium containing glycerine (most observations on a medium with a concentration of 4% glycerine). They noted increase in "degradation through pleomorphism". Our colonies, we felt, showed less pleomorphism as concentration of glycerine was increased with the possible exception of the 1% concentration. Microscopic changes due to glycerine will be the topic of a future paper.

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[1] Grigorakis, L., *Compt. rend. Soc. de biol.*, 1931, **108**, 94.

7405 P

Zone Phenomenon in Complement Fixation. Relation to Greater Sensitivity of Ice Box Type of Wassermann Reaction.

B. S. LEVINE. (Introduced by Lloyd Arnold.)

From the Clinical Laboratory of the Public Health Institute, Chicago.

Two hypotheses were recently presented for the explanation of the mechanism of zoning occurring in complement fixation—one by Levine,[1] the other by Eagle.[2] The two hypotheses rest on principles which possess no element in common. Therefore, I undertook a critical study of the subject matter. In this paper are presented in brief the results of the study of the theory of Eagle.

The latter can be briefly summarized as follows: Serum in concentrations greater than 1:25 effects an inhibition of complement fixation through the adsorption of fixation-preventing substances presumably present in negative serum.[3] These inhibiting substances are adsorbed by the antigen-antibody complexes after they had become fully organized. The adsorption is more intense the more negative a serum is, and corresponds roughly to the concentration of the serum protein. Hence, in weakly positive serums a more pronounced fixation of complement will occur in the higher serum dilution, and hence, the zone phenomenon. This same inhibition, Eagle states, is less marked at lower temperatures, and hence, in an indirect manner it is also responsible for the greater sensitivity of the icebox test.

Experimental studies were carried out as follows: (1) Complement and amboceptor titrations in the presence of high concentrations of negative serum; (2) complement fixation tests with Kahn antigen precipitated from positive and negative reactions[4] in

TABLE I.

The effect of the addition of some negative serum on the results of the complement titration.

	Cc. of 1-50 complement	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60
Diluent	Control tit'n saline only	* 100%	100%	100%	100%	90%	90%	50%	50%	25%	10%	10%	5%
	Plus 0.25 cc. negat. serum	100%	100%	90%	80%	50%	25%	25%	25%	5%	5%	Tr.	None

* Percentages indicate amount of unhemolyzed red cells.

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[1] Levine, B. S., *J. Inf. Dis.*, 1931, **48**, 189.

[2] Eagle, H., *J. Exp. Med.*, 1931, **53**, 615.

[3] Eagle, H., *J. Lab. Clin. Med.*, 1933, **18**, 725.

[4] Levine, B. S., *J. Lab. Clin. Med.*, 1929, **14**.

the presence of high negative serum concentrations; (3) the effect of the removal of the native antisheep amboceptor from negative serum and the bearing of this procedure on the assumption that the results of Eagle's original experiments were influenced by natural amboceptor.

TABLE II.

The effect of hemolysing and non-hemolysing serum on the results of the quantitative complement-fixation reaction.

	Cc. of positive serum	0.50	0.25	0.125	0.0625	0.0313	0.0156	0.0078	0.0039
Diluent	Saline	4*	4	4	4	4	2	1	Trace
	Serum A	4	4	4	4	2	1	Trace	None
	Serum B	4	4	4	3	2	2	2	1

* The numerals indicate degree of hemolytic inhibition in conventional terms. Serum A—hemolytic, Serum B—non-hemolytic.

TABLE III.

The effect of negative serum on the results of the quantitative complement-fixation reaction after the absorption of the native amboceptor.

	Cc. of positive serum	0.50	0.25	0.125	0.0625	0.0313	0.0156	0.0078	0.0039
Diluent	Saline	4	4	4	4	4	2	1	Trace
	Serum A_1	4	4	4	4	4	3	2	1
	Serum B_1	4	4	4	4	4	3	2	2

The results yielded by this critical study warrant the following conclusions: There is no evidence indicating the normal presence in negative serum of substances which inhibit the fixation of the complement by the antigen-antibody complexes either before or after they have become fully organized. It is erroneous to assume that some hypothetical substances are adsorbed by the antigen-antibody to a lesser degree at lower temperatures. One of the characteristics of adsorption in general is the fact that it progresses more slowly but reaches a higher maximum at lower temperatures.[5] It is precisely this characteristic of adsorption as a physicochemical phenomenon that is responsible to a large degree for the greater sensitivity of the icebox Wassermann. The validity of the quantitative Wassermann technic, although subject to some qualifications, is not affected by the results of Eagle's experiments. The latter were probably due to the native hemolytic properties of the negative serum used by that author. There is no discernible relationship between the cause of the zone phenomenon and that of the greater sensitivity of the icebox Wassermann. Hence, the hypothesis of Eagle is not tenable.

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[5] Ware, J. C., The Chemistry of the Colloidal State. John Wiley and Sons, Inc. New York, 1930.

7406 P

A Simple Volumetric Method for Determination of Fat in Blood Plasma*

NAT N. ALLEN. (Introduced by W. E. Petersen.)

From the Division of Dairy Husbandry, University of Minnesota.

Investigations of changes in the fat content of the blood have been handicapped greatly by the tedious methods available for determination of blood lipids. The method described in this paper is easily and quickly carried out and is thus adapted to work requiring the analysis of large numbers of samples. Duplicate samples check closely, and composite samples agree well with calculated values, indicating that the method is satisfactory for comparative studies.

The design and approximate dimensions of the test bottle used for

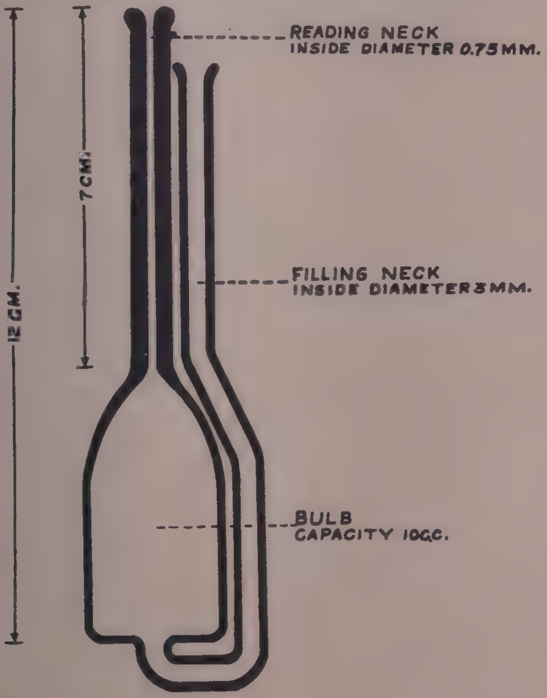

Fig. 1.

Cross section of test bottle used for determination of fat in blood plasma.

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* Paper No. 1262 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

the determination are shown in Fig. 1. An alkali reagent developed by Petersen and Herreid[1] for the purpose of testing buttermilk by a modified Babcock procedure is employed to free the fat from the blood plasma. This reagent consists of 110 gm. of sodium carbonate and 200 gm. of sodium salicylate dissolved in water and made up to a volume of one liter, to which are then added successively 30 cc. of 50% sodium hydroxide and 100 cc. of normal butyl alcohol. The samples should be tested in duplicate if the amount of blood plasma available permits.

Three cc. of blood plasma are measured into the bottle by means of a pipette. A very gentle suction applied to the opening of the reading neck will cause the sample to flow into the bottle readily. Five cc. of reagent are then added in the same manner and thoroughly mixed with the plasma by allowing air to be drawn through the mixture by the suction used in filling the bottle. The filling neck is then closed tightly by means of a small stopper, the bottle is placed in a water bath, and digested for 20 minutes at 180° to 190° F., with occasional shaking.

The stopper is then removed and the bottle is centrifuged for 5 minutes. A speed of 800 revolutions per minute in a centrifuge with a diameter of 18 inches has been found to be sufficient. Water at 160° to 180°F. is then added until the bottle is filled almost to the base of the reading neck. After centrifuging for about 2 minutes water is again added until the filling neck overflows. Since the reading neck is about 1 cm. longer than the filling neck, this automatically brings the fat column to the proper level for reading. The addition of water to the sample is greatly facilitated by use of a long slender cannula, such as a hypodermic needle of large diameter, connected to an elevated container of hot water by means of rubber tubing equipped with a pinchcock. The cannula is inserted well into the filling neck when water is being added, to avoid forcing bubbles of air into the bottle. The sample is again centrifuged for 2 minutes and removed to a water bath at 140°F. for 5 minutes before reading. Before placing in the water bath, a small wooden plug is inserted tightly into the opening of the reading neck to prevent any shifting of the position of the fat column.

The length of the fat column is measured by means of calipers, and a calibration factor is applied to convert the reading to mg. per 100 cc. The reading neck is calibrated by means of mercury and the factor for a 3 cc. sample is secured by the formula:

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[1] Petersen, W. E., and Herreid, E. O., *Minn. Agr. Exp. Sta. Tech. Bul.*, 1929, 63.

Calibration Factor

$$= \frac{\text{Vol. of neck in cc. per mm.} \times \text{specific gravity of fat} \times 1000 \times 100}{\text{size of sample in cc.}}$$

$$= \frac{\text{Vol. of neck in cc. per mm.} \times 0.93 \times 1000 \times 100}{3}$$

$$= \text{Vol. of neck in cc. per mm.} \times 31{,}000$$

The length of the fat column in millimeters is multiplied by this calibration factor to convert the reading directly to milligrams per 100 cc. The figure used for specific gravity, 0.93, is the average specific gravity of the fat recovered from bovine blood by the method described in this paper, at a temperature of 140° F., as found by the pycnometer method for 13 different samples. By merely changing the specifications of the test bottle illustrated in Fig. 1, a bottle of similar design but smaller dimensions has been constructed for testing samples of only 1 cc. of plasma.

In addition to the speed and ease with which this method is carried out, it has the further advantage of requiring no filtrations or extractions which might result in a loss of material. The fat as recovered is presumably in the same chemical state as when it is in the blood plasma, the only difference being that it is freed from the emulsifying agents. The only factor necessary for securing the final reading on the sample is that necessary for converting the volumetric measurement to terms of weight.

The work indicates that phospholipids and free fatty acids are not recovered with the fat. The cholesterol of the blood plasma is apparently recovered with the fat, as shown by applying tests for cholesterol to the fat and to the non-fat residue of the test. This probably is largely responsible for the relatively high specific gravity of the blood fat. More extensive work is necessary to completely establish these facts in connection with the applicability of the method for absolute determinations.

7407

Shift of Body Fluids in Severe Burns.

HENRY N. HARKINS. (Introduced by Edmund Andrews.)

From the Douglas Smith Foundation and the Department of Surgery, University of Chicago.

Blalock,[1] Parsons and Phemister,[2] and others have shown that in traumatic shock and in severe burns there is a marked shift of fluid from the blood into the injured tissues. This fluid shift has been measured in the case of burns by burning one lateral half of the animal and then after midline sagittal section, measuring directly the difference in weight of the 2 halves. The amount of fluid shift has been found sufficient to explain the resultant shock. This method allows only one determination of the amount of shift in each animal. Because of variation in the extent of the burn and in the reaction of individual animals, a complete record of the rate of shift of fluid will not be obtained.

In all experiments completely anesthetized animals who suffered no pain whatsoever were used and no burned animals were allowed to recover.

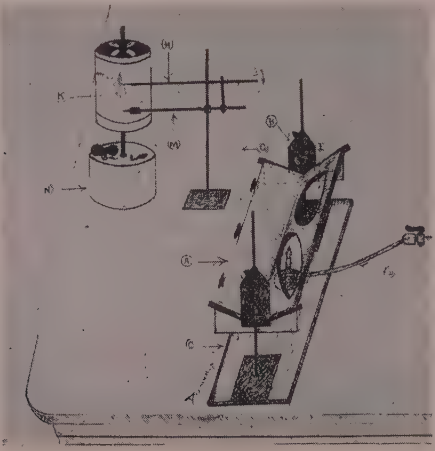

FIG. 1.
A—Trough on which animal is placed, B—steel end plates with holes for knife-edge suspension, C—base, D—tube to bunsen burner, E—hole for burn, G—recording thread, H—marker, K—smoked drum, M—time marker, N—drum stand.

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[1] Blalock, A., *Arch. Surg.*, 1931, **22**, 610.

[2] Parsons, E., and Phemister, D. B., *Surg. Gynec. and Obst.*, 1930, **51**, 196.

The author devised the apparatus shown in Fig. 1. An animal placed on this apparatus is in a state of balance and any lateral shift in fluid will cause a tipping of the apparatus and resultant change in the position of the marker on the kymographic drum. When one-half of the animal is burned, the fluid shift to the burned side results in such a tipping and this in turn is recorded on the drum as a tracing.

Large rabbits or small dogs anesthetized with barbital and ether were used in all experiments. The animal was completely encased in a plaster cast except an opening on one side for the fore leg, axilla and upper chest and another opening for the lower abdomen and hind leg, which were burned. The tracing was continued until death. The animal was then divided into 2 lateral halves and weighed, the head, tail, viscera and spinal column being discarded.

The results in 4 animals burned on the balanced apparatus are given in Table I. It is seen that in general about half of the total shift occurred in the first hour. In the first 2 experiments the total shift was obtained only approximately by placing weights on the apparatus at a distance from the fulcrum corresponding to the center of the burned area until the apparatus was again in balance. The accuracy of this approximation may be gauged from the observation that in the third experiment the approximation gave 55 gm. and the direct weighing 89 gm. shift and in the fourth experiment the two methods gave 145 and 130 gm. respectively.

TABLE I.

			Fluid shift in % of total shift												
No.	Animal	Wgt. kilos	10′	20′	30′	45′	60′	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	Time of death in hrs.	Total shift in gm.	Total shift as % body wt.
1	Rabbit	3.3	—	—	—	—	—	—	—	83	—	100	5½	24	0.7
2	Dog	5.0	0	25	32	36	41	57	70	81	87	97	6½	170	3.4
3	Dog	4.0	0	17	25	33	42	75	100	—	—	—	3½	89	2.2
4	Dog	5.0	24	—	56	60	64	72	84	88	96	100	5½	130	2.6

Control curves obtained by burning dead animals and by burning plaster of Paris showed only a slight shift of weight to the unburned side.

7408 P

Effect of 2-6 Dinitrophenol on Yeast Respiration.*

J. FIELD 2nd, A. W. MARTIN AND S. M. FIELD.

From the Department of Physiology, Stanford University.

Undissociated 2-4 dinitrophenol (alpha-DNP) stimulates yeast respiration[1, 2, 3] and fermentation.[1] We have undertaken a survey of the action of the isomers of this substance, the first studied being 2-6 dinitrophenol (beta-DNP). The experimental procedure has been described elsewhere.[1, 2]

Beta-DNP resembles alpha-DNP in that only the undissociated acid affects yeast metabolism. The action is less intense, in that optimal doses of alpha-DNP cause approximately 100% increase in the rate of oxygen consumption, while the beta form raises the respiratory rate by about 50% in optimal doses. Similar relations have been demonstrated in the capacities of these substances to induce hyperthermia in the pigeon.[4] The optimal concentration of beta-DNP at pH 5.4 and at pH 6.0 in glucose phosphate solutions is about 1.4 mg. of the undissociated acid per liter.

Like alpha-DNP, beta-DNP rapidly induces either stimulation or inhibition, depending upon concentration. The effect develops within 5 minutes.

Inhibition of respiration by beta-DNP is reversible, as is the similar inhibition by alpha-DNP.

A slight further increase over the beta-DNP optimum can be evoked by addition of alpha-DNP. Addition of optimal concentration of beta-DNP to yeast suspensions in which the alpha-DNP concentration is optimal causes a decrease in the alpha-DNP effect. Further studies on these interrelations are in progress.

Like alpha-DNP, the action of beta-DNP does not depend upon the presence of glucose, but unlike alpha-DNP the usual stimulation pattern in phosphate buffer at pH 5.4 is not obtained in acetate buffer at pH 5.4.

Studies on the action of other isomers are now in progress.

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* Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

[1] Field, J. 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

[2] Field, J. 2nd, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.*, in press.

[3] Ehrenfest, E., and Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 318.

[4] Tainter, M. L., and Cutting, W. C., *J. Pharm.*, 1933, **49**, 187.

7409 P

Effect of 2-4 Dinitrophenol on Inhibition of Yeast Respiration by Cyanide.*

J. FIELD 2nd, A. W. MARTIN AND S. M. FIELD.

From the Department of Physiology, Stanford University.

Tainter and coworkers[1, 2] and others[3] have shown that 2-4 dinitrophenol (alpha-DNP) stimulates respiration in animals by direct action on the tissue cells. We have shown previously that this substance stimulates or inhibits respiration and fermentation of yeast (*Saccharomyces cerevisiae,* Race F) depending upon the concentration of the undissociated acid form.[4, 5]

The experimental procedure used in the present study has been described elsewhere.[4, 5]

Treatment of yeast suspended in glucose phosphate media with M/1000 NaCN of the same pH reduces respiration to about 14% of the control rate. If alpha-DNP be now added in a dose which causes optimal stimulation of the cyanide-free yeast, *i. e.,* in amount giving a free acid alpha-DNP concentration of 0.64 mg. per liter at pH 6.8,[5] it further depresses the respiration of the yeast poisoned with cyanide.

However, the respiration of yeast poisoned with cyanide can be stimulated by alpha-DNP, provided that the concentration of the undissociated acid form of this agent be approximately 0.032 mg. per liter, *i. e.,* about 1/20 of the optimal dose for unpoisoned yeast. Under these conditions the respiratory rate of the poisoned yeast may be increased by about 20%. This relation has been demonstrated at pH 5.9 and pH 6.8. Further experiments on the action of alpha-DNP on respiration of organisms and tissues poisoned by cyanide are in progress.

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* Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

[1] Cutting, W. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1268.

[2] Hall, V. E., Field, J. 2nd, Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.,* 1933, **106**, 432.

[3] Magne, H., Mayer, A., and Plantefol, L., *Ann. Physiol. Physicochim. Biol.,* 1931, **7**, 269.

[4] Field, J. 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

[5] Field, J. 2nd, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.,* in press.

7410 P

Some Reactions of Ammonolyzed Epinephrine.

R. G. ROBERTS, H. C. COBLENS, W. E. DYKO AND H. H. COHEN.
(Introduced by William S. Hoffman.)

From the Department of Physiological Chemistry, Chicago Medical School.

Previous work[1] on certain nitrogenous substances in liquid ammonia has shown that some of them tend to ammonolyze with changes in their physical, chemical, and biological properties. Ammonolyzed edestin was reported[2] not to have given an anaphylactic reaction with a guinea pig previously sensitized to the untreated edestin. Recent observations on changes in some physical constants of certain amino acids in liquid ammonia indicate an ammonolysis. Epstein, Gunn and Virden[3] have shown changes brought about in the physiological activity of phenylethylamines by methylation. These facts, in addition to the observations of Kraus and White[4] that the hydroxyl groups of alcohols and phenols are quite reactive in liquid ammonia and to the report of Roberts and Miller[5] that the phenolic group of tyrosine seems to be acidic in liquid ammonia have led us to believe that epinephrine could be ammonolyzed and thus altered in its physiological activity.

The chemical treatment consisted of adding 60 mg. of Parke, Davis adrenalin to 150 cc. of liquid ammonia contained in a Dewar flask connected by gas-tight stoppers and tubing to a mercury seal allowing the ammonia to vaporize without admitting moisture to the adrenalin. The liquid ammonia, previously dried over sodium by the method of Fernelius and Johnson, boils off in about 24 hours, and the drug is ready for use. The yellowish adrenalin base darkens to a reddish-brown on standing in liquid ammonia, and its solubility in water and in glycerine decreases.

The flaky material is powdered, and used in the form of an aqueous sol. With glycerine used as a vehicle, the suspension is more uniform, and the base has less tendency to settle out. When water is used, the injection is made at once since the ammonolyzed base hydrolyzes on standing. With glycerine, a pressor activity characteristic of the new compound was obtained after 30 days, but after standing for 6 months an adrenalin curve was again obtained on injection.

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[1] Miller and Roberts, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 533.

[2] Private communication from E. Gebauer-Fuelnegg.

[3] Epstein, Gunn and Virden, *J. Physiol.*, 1932, **76**, 224.

[4] Kraus and White, *J. Am. Chem. Soc.*, 1923, **45**, 768.

[5] Roberts and Miller, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 821.

Dogs were the only animals used, and the drugs were administered by injection into the femoral vein. Either pentobarbital or ether were used as anesthetics, and water or glycerine were the only vehicles. When the ammonolyzed adrenalin, usually in dilution of 1:20,000, was injected, a curve was obtained that in a general way resembled the curve obtained from the untreated adrenalin control, but the initial rises were less rapid, the peak of the secondary curve was more sustained and lengthened giving an increased time above the base line of about 50% in general and the heart beat was markedly increased. The compound's activity is decreased after reduction with sodium.

Alles[6] in studying the pressor and toxic effects of phenylethyl and phenylisoprophylamine decided that the side chains did not affect the intensity factor, but did affect the duration factor although the introduction of hydroxyl groups into the phenyl group also played a part in duration as well as in intensity.

In comparing the chemical structure and pressor activity of known pressor substances, such as epinephrine, synephrine, tyramine and ephedrine, it would seem that blocking the adjoining catechol hydroxyl groups of epinephrine by methylation or ammonolysis would prolong its pressor effect. We observed no toxic effect from ammonolysis as has been reported from methylation. It seems most likely to us that the hydroxyl groups of the phenyl radical have been converted into ammonia addition products rather than into amines and, possibly the hydroxyl of the side chain and the trivalent nitrogen have been affected. Possibly treatment in liquid methylamine would give results that would be more satisfactory than either methylation or ammonolysis.

We plan to make a study of the nitrogen distribution in the ammonolyzed adrenalin, and to complete other work that we have done on the ammonolysis of ephedrine, synephrine, atropine, pilocarpine, ergotamine, and insulin.

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[6] Alles, *J. Pharm. and Exp. Therap.*, 1933, **47**, 351.

7411 P

Recent Observations on Thyroxine and Allied Compounds.

W. O. THOMPSON, S. B. NADLER, S. G. TAYLOR, III, AND P. K. THOMPSON. (Introduced by A. B. Hastings.)

From the Department of Medicine, Rush Medical College, the Presbyterian and Cook County Hospitals, Chicago, and the Department of Biochemistry, Tulane University, New Orleans.*

In an effort to determine whether the formation of the comparatively soluble disodium salt accounts for the well-marked increase in basal metabolism recently observed by Thompson *et al*[1] following the oral administration of thyroxine in alkaline solution, we have prepared a disodium salt of thyroxine according to the method of Kendall[2] and have determined its calorigenic action in patients with myxedema. In one patient a single oral dose in distilled water of 15.1 mg. of the product containing 6.5 mg. of iodine, produced an increase in basal metabolism only from —29% to —21%, and in another patient the same dose produced an increase only from —26% to —20%. These increases are about the same as those produced by single doses of the monosodium salt containing the same amount of iodine and only about ⅓ as great as those produced by thyroxine in alkaline solution. This small effect may be explained in at least 2 ways: (1) The method as used by us may not actually yield the disodium salt. (2) A large part of the disodium salt may hydrolyze when treated with distilled water to form sodium hydroxide and thyroxine. Although the disodium salt is said to be soluble to the extent of 4% and the monosodium salt only slightly soluble, the well-marked effect of thyroxine in alkaline solution by mouth must not be attributed to the formation of the disodium salt without more evidence.

N-acetyl thyroxine, prepared according to the method of Kendall and Osterberg,[3] has been given intravenously in alkaline solution to 3 patients with myxedema in doses of from 10.5 mg. to 31.5 mg., containing from 6.5 mg. to 19.5 mg. of iodine; subcutaneously, suspended in 10% glucose, to 2 patients with myxedema in doses of 10.5

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* We are greatly indebted to Dr. Frederick Tice and Dr. Karl A. Meyer for the privilege of making some of these observations in the Cook County Hospital.

[1] Thompson, W. O., Thompson, P. K., Dickie, L. F. N., and Alper, J., *Arch. Int. Med.*, 1933, **52**, 809.

[2] Kendall, E. C., "Thyroxine." New York, The Chemical Catalog Company, Inc., 1929.

[3] Kendall, E. C., and Osterberg, A. E., *J. Biol. Chem.*, 1919, **40**, 265.

and 21.0 mg. respectively; and orally to one patient with myxedema in a dose of 10.5 mg. The patient who received 31.5 mg. intravenously showed a slight but fairly prolonged increase in basal metabolism of from —41% to —35%. If this slight increase may be considered definite, then this compound when administered intravenously in alkaline solution produces only about 1/16 of the effect of thyroxine. When given subcutaneously in suspension, the effect was greater and amounted in one patient to about ¼ and in another to about 1/6 of the effect of thyroxine given intravenously. These data suggest that the amino group is important for the calorigenic action of thyroxine.

By proteolytic digestion of desiccated thyroid according to the method of Harington and Salter,[4] we have prepared a peptide of thyroxine containing 2.5% nitrogen and 48% iodine, with a nitrogen:iodine ratio of 0.48:1. This peptide was insoluble in distilled water but soluble in sodium hydroxide. When suspended in distilled water and administered by mouth to patients with myxedema, it produced only a slight increase in basal metabolism, which was about the same as that produced by the monosodium salt in doses containing the same amount of iodine. When administered by mouth in alkaline solution it produced 3½ times as much effect as when given suspended in distilled water. In an alkaline solution its effect was about 4/5 as great as that of thyroxine injected intravenously in an alkaline solution, and slightly greater than those of oral administration of desiccated thyroid and an alkaline solution of thyroxine in doses containing the same amount of iodine.

7412 P

Antistreptolysin Content of Blood Serum of Children. Its Significance in Rheumatic Fever.

MAY G. WILSON, G. W. WHEELER AND M. M. LEASK. (Introduced by O. M. Schloss.)

From the Department of Pediatrics, Cornell University Medical College and the New York Hospital.

Todd[1a] described a method of titrating antistreptolysin in blood serum by which a diagnosis of a preceding hemolytic streptococcus infection may be made. Coburn and Pauli[2] considered the antistreptolysin titre of 200 units or higher at the onset of rheumatic fever as immunologic evidence that the hemolytic streptococcus initiates the disease. Myers and Keefer,[3] however, obtained an average of 200 units in normal subjects with a higher average (500 units) in rheumatic fever.

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[4] Harington, C. R., and Salter, W. T., *Biochem. J.*, 1930, **24**, 456.

[1] (a) Todd, E. W., *J. Exp. Med.*, 1932, **55**, 267. (b) Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248.

The majority of these observations were made in adults. They represent either single determinations or repeated tests over a short period and give a wide normal range of from 50 to 500 units.

In view of these findings it seemed of interest to obtain information as to the antistreptolysin titre in children of various ages, including: (1) Subjects apparently well; (2) Convalescents following streptococcus and miscellaneous infections; and (3) Rheumatic children during active and inactive periods.

Method. Todd's technic[1b] was closely followed. A standard horse serum and patients' serums of known titre were used as controls in each series of tests.

Control series included 150 subjects from 3 months to 16 years of age; a group of 67 subjects in apparent health (no history of streptococcus infection); 51 convalescing from miscellaneous infections (varicella, pertussis, pneumonia); and 32 convalescing from hemolytic streptococcus infections (scarlet fever, erysipelas, respiratory infections). The rheumatic series included 133 subjects under weekly observation for from 9 to 12 months; 41 patients with active rheumatic fever (chorea, carditis, arthritis); and 92 subjects in the inactive stage.

Observations are summarized in Table I.

The observations on sixteen mothers and newborn infants (2 to 8 days of age) are of particular interest. In the majority of the tests the titre for mother and infant were the same; in the remaining the infants were consistently higher. Repeated determinations were made at 2, 3, and 4 weeks; the mothers' sera remained at the same level, while the infants' sera progressively diminished, approaching the average level secured for children 3 months to 3 years old. These observations would indicate placental transmission of this antibody.

An analysis of the average antistreptolysin titre for each age group in the normal series indicates a definite correlation with age, the lowest values being obtained in the 3 month to 3 year group.

A comparison of the average titres for normal subjects (200

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[2] Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 651.

[3] Myers, Walter K., and Keefer, Chester S., *J. Clin. Invest.*, 1934, **13**, 155.

TABLE I.
Antistreptolysin Titre of Sera in Children.

			Control Series							Rheumatic Series	
			Normals			Infections				Active	Inactive
						Non-Streptococcus		Streptococcus			
Antistreptolysin	Mother	New-Born	¼ to 3 yrs.	3-5 yrs.	6-16 yrs.	¼ to 3 yrs.	3-16 yrs.	¼-3 yrs.	3-16 yrs.	6-16 yrs.	6-16 yrs.
Total Subjects	16	16	11	8	48	34	17	22	10	41	92
*Average Units	*50*	*56*	*10*	90	*200*	50	125	166	*500*	*250*	*200*
,, N.D. cc.	.02	.02	0.1	.012	.005	.02	.008	.006	.002	.004	.005
Above Average %	31	43	18	37	25	32	41	40	40	27	35
Below ,, %	50	31	0	62	69	68	47	56	40	54	53
Range in Units	25-83	—10-100	—10-20	10-333	20-1000	10-200	10-350	10-500	10-1000	50-1600	33-1000
Mode ,, ,,	50	25-62	—10	50	*166*	144	144	333	*500*	*200*	*200*

*The average titre for each group includes single representative determinations for each subject. A total of 679 determinations was made.

units) and rheumatic patients, active or inactive (250-200 units), of the same age group shows no significant difference. The average value in subjects with preceding streptococcus infections (500 units) was definitely higher.

The wide range of titres, from 20 to 1000 units, observed in both the control and rheumatic series indicates the importance of knowing the antistreptolysin level of each individual for a control period before attaching any significance to a single determination. High titres, 333 to 1000 units, were maintained over periods of from 5 to 9 months in normal and in rheumatic subjects who were apparently well. It is also of interest that higher values were obtained during the winter and spring months.

A significant rise in the antistreptolysin titre was frequently noted in rheumatic subjects, unaccompanied by rheumatic activity. Only 20% of the subjects who developed rheumatic fever during the period of observation exhibited a rise in titre above their level during the control period. The rise in titre was not directly related to type, severity or duration of the rheumatic activity.

The evidence presented in this study does not indicate that the antistreptolysin content of the serum of children with rheumatic fever is of specific etiologic significance.

7413 C

Quantitative Colorimetric Estimation of Morphine in Biological Fluids by the Iodoxybenzoate Method.*

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Development of a satisfactorily accurate quantitative application of the usual color reactions for morphine has been retarded because of their general lack of (a) proper selectivity for this single opium alkaloid, (b) permanence of hue produced or (c) sufficient sensitivity. In addition such reactions are in most instances dependent to an undesirable degree on the temperature and pH of the test solution and the presence of commonly occurring contaminants. This situation is not surprising since most of these reactions are supposedly due to the formation of complex unstable chromogenic substances. A more ideal quantitative test should be the stoichiometric conversion of morphine to a simple colored form.

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* Published with the permission of Mr. W. G. Walker, Chief, Division of Narcotic Enforcement, Department of Penology, State of California.

Leake[1] suggested that such an effect could be obtained for many substances by reacting their free phenolic groups with an aqueous solution of ammonium iodoxybenzoate, the oxidative powers of which had been studied in another regard by Loevenhart[2] and others. Morphine rapidly develops the same color when treated with this agent as is produced by slow spontaneous oxidation of morphine solutions in air, and it was recommended[1] that this reaction be used not only as a qualitative test to differentiate morphine from codeine, heroin, dionin and apomorphine, but also as the basis of a quantitative colorimetric estimation. We have found that when 25 to 50 mg. of powdered ammonium iodoxybenzoate are added to 5 to 10 cc. of 0.005 M to 0.02 M aqueous solutions of morphine salts sufficient agreement with Beer's Law holds so that colorimeter readings yield results accurate to ±3% if the comparison with a standard is made after 30 minutes are allowed for full development of the color. Spectroscopic examination of color absorption by the treated solutions reveals that in concentrations higher than 0.03 M extinction extends further toward the yellow band than with the lower concentrations, which is represented grossly by a change in shade from orange to garnet and considerable deviation in colorimetric measurements unless a suitable filter is used. In practice it is simpler to adjust the concentration of the unknown solution after one approximation, which also serves the purpose of a qualitative test, to about 0.01 M or 0.005 M and then compare it with a standard accurately made to 0.01 M or 0.005 M. The reaction is sufficiently sensitive to yield fair results down to 0.0015 M morphine solutions, but the error increases somewhat at the lower limits.

Estimations in serum and urine are not as satisfactory, the error amounting to ±7% and ±5% respectively. Trichloracetic acid filtrates may be used or extraction of ammoniacal solutions of serum or urine containing morphine salts with $CHCl_3$ and subsequent conversion of the extractive to a water-soluble salt may be carried out to permit a more accurate determination in distilled water. Sucrose and lactose do not interfere with the quantitative estimation, thus allowing direct determinations on syrups and solid preparations contaminated with milk sugar. Semi-quantitative estimations may be carried out in oil solutions as well. The nature of the anion of the morphine salt has no influence on the color produced. Heavy metals precipitate iodoxybenzoate and so interfere with the test. The amount of iodoxybenzoate used has no effect on the intensity of the color produced, but maximum intensity is reached in a shorter time if larger amounts are used; excessive amounts cause a troublesome precipitate, however. Standards once prepared remain constant as to intensity of color for long periods. Attempts to concentrate the color in test solutions of concentrations less than 0.001 M morphine by precipitating it with $Al(OH)_3$, $Zn(OH)_2$ or kaolin have not been successful, and since the method is sensitive to less than 0.1 mg. of morphine per cc. no further attempts to increase the sensitivity have been made.

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[1] Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 148.

[2] Loevenhart, A. S., and Grove, W. E., *J. Pharmacol. and Exp. Therap.*, 1911, **3**, 101.

Since most of the illicit morphine confiscated in California is in the form of morphine hydrochloride, a rapid check accurate to ±0.5% can be made on the customary extraction-titration methods used routinely for such evidence by following the colorimetric morphine determination with a microchloride determination. If the chloride determination falls within the calculated value obtained to ±3% colorimetrically, it is safe to assume the corresponding amount of morphine is present to within ±0.5% because of the rarity of diluents having a similar Cl content which at the same time themselves react with iodoxybenzoate. The economy of time and amount of substance used in the test is a notable advantage over the older methods.

Summary. The qualitative iodoxybenzoate test of Leake has been found suitable for quantitative adaptation to the estimation of morphine. Methods for determining morphine in biological fluids and a rapid method of accurately checking the morphine content of morphine hydrochloride are suggested.

7414 P

Nature of the Hemolytic Agent in Previously Irradiated Solutions of Eosine.

H. F. BLUM AND C. R. SPEALMAN.

From the Division of Physiology, University of California Medical School, Berkeley.

One of us[1] demonstrated that red blood cells are hemolyzed by solutions of fluorescein dyes which have been previously irradiated with the visible portion of sunlight. Finding that such solutions contained a small concentration of peroxide,[1] and for reasons which seemed consistent with the then known facts of photodynamic action, we assumed that the hemolysis was probably due to the activity of this peroxide, which we suspected of being an organic peroxide. Recently,[2] however, we have offered evidence to show that this peroxide is H_2O_2.

We have determined the quantity of peroxide and dye in solutions which are just capable of producing hemolysis, and have found that mixtures of eosine and H_2O_2 do not produce hemolysis at nearly such low concentrations of these reactants as do irradiated eosine solutions. Table I indicates this discrepancy.

Furthermore, in most cases, we have found that when $Na_2S_2O_3$ is added to the irradiated solutions in concentrations just equivalent to the concentration of H_2O_2 present, hemolysis is not abolished nor in most cases perceptibly diminished.

TABLE I.
Concentrations of eosine and H_2O_2 in irradiated and non-irradiated solutions just producing hemolysis.

Eosine millimols per l.	H_2O_2 milli-equivalents per l.	Increased hemolytic activity	Increased hemolytic activity per milli-equivalent H_2O_2	Quantity of eosine bleached millimols per l.
		Non-Irradiated Dye.		
5	6	2.5	0.4	
2.5	8	5	0.6	
0.6	11	21	2	
0.15	45	83	2	
0.07	90	166	2	
		Irradiated Dye.		
0.43	0.17	29	170	0.07
0.9	0.1	14	140	0.1
0.475	0.3	13	42	0.025
0.34	0.1	17	170	0.17

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[1] Blum, H. F., *Biol. Bull.*, 1930, **58**, 224.

[2] Blum, H. F., and Spealman, C. R., *J. Phys. Chem.*, 1933, **37**, 1123.

An amount of bleaching of the dye solutions occurs which is detectable spectrophotometrically; the quantities are indicated in Table I. We have been forced to conclude that the products of this bleaching, and not peroxide are responsible for at least the greater part of the increased hemolytic properties of our solutions. Further experiments are in progress.

7415 P

Observations on the Treatment of Infertile Rabbits with Antuitrin "S".

PAUL D. ROSAHN, HARRY S. N. GREENE AND C. K. HU.

From the Laboratories of The Rockefeller Institute for Medical Research.

Prolonged experience with a large breeding colony of rabbits has revealed that a small proportion of sexually mature females repeatedly fail to become pregnant after being served by known fertile bucks. In general, such does are in excellent physical condition and show no external evidence of disease, although a few have a tendency to obesity characterized by a shoulder girdle adiposity and an abnormal accumulation of fat in the abdomen. Recently, certain of these persistently infertile does carried genetic factors which were under investigation, and progeny from them were desired in order to carry on the genetic studies. Accordingly, they were submitted to different therapeutic procedures, with a view to developing a method which would render them fertile. Since all the animals were of value because of their genetic constitution, operative interference and experiments hazardous to life were of necessity avoided. The present report is concerned with one of the methods employed in the treatment of these does.

In the rabbit, ovulation occurs about 10 hours after copulation. It was thought that the threshold necessary to induce ovulation in refractory females was higher than the stimulus afforded by the act of coitus. A simple method of augmenting the copulation stimulus lay in the observation of Friedman[1] that the parenteral administration of human pregnancy urine induces ovulation in the rabbit. The method employed in the present investigation was to inject a single dose of 40 Rat Units per kilo body weight* of Antuitrin "S" into the marginal ear vein of the doe. The time of mating with a known fertile buck with reference to the injection varied from 22 hours before to 24 hours after the administration of Antuitrin "S". After a lapse of 10 days, when clinical examination determined the existence of a non-pregnant state, the procedure was repeated with a few does. The investigation was begun on January 18 and continued to March 19, 1934. The control period of matings with which the experimental results were compared began in the middle of September, 1933, and continued up to the time the animal was treated, thus ranging from 4 to 6 months. It was during this interval that repeated matings established the infertility of the doe and determined her availability for this study.

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[1] Friedman, M. H., *Am. J. Physiol.*, 1929, **90**, 617.

* This dose was suggested by the work of Snyder, F. F., *Bull. Johns Hopkins Hosp.*, 1934, **54**, 1.

Forty-three trials were made on 27 rabbits, each trial consisting of the administration of Antuitrin "S" plus service by a fertile buck. Six deaths to be discussed later followed the administration of the pregnancy urine principle. These occurred in 4 animals being observed for the first time and in 2 animals each of which had been tested on one previous occasion. The results are, therefore, based on 37 trials with 23 animals.

Nine pregnancies and 28 infertile matings resulted from the 37 trials. During the control period, the 23 animals in the group had been served on 122 separate occasions with 9 pregnancies resulting. The occurrence of pregnancy in relation to the Antuitrin "S" treatment was significantly more frequent than the incidence of pregnancy during the control untreated period ($\chi^2 = 8.05$, $P = 0.01-$, significant). The series was divided into 2 groups depending on the relation of the Antuitrin "S" treatment to the time of mating. In 9 trials the drug was given from 20-24 hours before the mating—no pregnancies resulted. Twenty-eight injections within a 2-hour period before to 22 hours after mating resulted in 9 pregnancies. Thus more pregnancies were noted when the Antuitrin "S" was given from 2 hours before to 22 hours after mating than when it was injected 20-24 hours before ($\chi^2 = 3.85$, $P = 0.05-$, probably significant). Nine pregnancies occurred in the group of 28 trials on 16 animals receiving the drug within 2 hours before to 22 hours after service. These 16 animals had been mated 85 times during the control period and only 2 pregnancies resulted. The relation of pregnancy and treatment in this group was very highly significant ($\chi^2 = 21.56$, $P = 0.01-$, significant). In this treated group 9 of the 16 animals became pregnant after an average of 1.7 matings per animal, whereas in the untreated control period pregnancy was noted twice in only one of these 16 rabbits following a mean of 5.3

matings per animal ($\chi^2 = 9.32$, $P = 0.01-$, significant). One persistently infertile doe did not become pregnant when mated in conjunction with treatment. The next mating 10 days later without treatment resulted in a pregnancy, the first in 6 attempts, and this was followed by a second pregnancy also without treatment. In 2 instances, does which were non-pregnant after treatment, became pregnant following a subsequent administration of the drug plus service.

Five of the 6 deaths took place from 2-18 hours after the administration of Antuitrin "S" and were definitely associated with the treatment. The sixth death occurred 7 days after the injection and may or may not be related to the drug. All of these animals showed evidence of an acute physiological upset. Congestion and hemorrhage of the subcutaneous vessels particularly in the region of the mammary glands, and congestion and hemorrhage of the thymus, vagina and uterus were frequent findings. The exact cause of these deaths is not known at present, but the common feature of their occurrence in abnormally obese animals suggests a violent endocrine and vasomotor crisis precipitated by the drug.

The results suggest that one factor involved in the sterility of certain does is their failure to ovulate after coitus, and that this failure may be remedied by the administration of pregnancy urine principle. It is possible that repeated injections and doses larger than 40 Rat Units per kilo might cause ovulation in those does which were refractory to the treatment described herein. There is some evidence to support the view that the stimulating effect of Antuitrin "S" may lower the ovulation threshold, so that a subsequent mating after the lapse of many days and even weeks, might have a fertile result without its adjunct at the time of the succeeding matings.

7416 P

Observations on Vitamin Treatment of Reproductive Abnormalities in the Rabbit.

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From the Laboratories of The Rockefeller Institute for Medical Research.

The reproductive activities of certain rabbits in our breeding colony have been observed on repeated occasions to be abnormal. These abnormalities may be classified, for present consideration, in 3 phases, namely, infertility, desertion or neglect of the young, and cannibalism. Observations indicated that some of these conditions were associated with nutritional disorders and it had been found that some improvement could be obtained by a general increase in vitamin intake. Accordingly, an attempt was made to correct the abnormalities of a selected series of animals by the administration of vitamin concentrates singly or in various combinations. The results thus far obtained have been highly encouraging and these form the basis of the present communication.

A group of 42 rabbits was selected for this study. These included 38 females and 4 males of pure bred and hybrid stocks, chiefly Rex, Belgian hare, English, Havana, Dutch, Polish, Himalayan and a highly inbred Albino family. In each case previous observations had indicated that the reproductive behavior was abnormal. Following vitaminization for various periods of time, the treated animals were mated with known fertile animals and the results were measured by subsequent observations made with regard to the particular abnormality presented. In all cases except one, dried brewer's yeast, representing the B complex was used either alone or in combination with halibut liver oil or viosterol or with both. Halibut liver oil alone was employed in one case. All vitamin concentrates were administered by hand. A few preliminary tests were made during the Winter of 1932-33 and the Spring of 1933, but most of the experiments were carried out between December, 1933, and February, 1934, inclusive. The test animals have lived indoors from birth and were distributed among normals in several rooms, and all the rabbits of the colony were fed a standard diet.

Nineteen does and 4 bucks were infertile, as indicated by failure to reproduce upon repeated matings. Thirteen does either frankly deserted or made no attempt to nest or to raise the young. Six does destroyed their young at birth or within a few days after birth and deliberately ate them.

As a result of vitaminization, the abnormalities were corrected in a significant number of instances. The period of vitaminization varied from a minimum of 4 days to a maximum of 2½ months; most of the animals were treated a little over a month. Of the 19 persistently infertile does, 9 became pregnant after vitaminization. Of these 9, 5 were pregnant upon the first mating and 4 required repeated matings—2 mated twice, 1 mated three times and 1 mated 4 times—before a fertile mating was obtained. These results as compared with those from matings made during the 3 months prior to the treatment were highly significant as shown by the χ^2 test. Before vitaminization there were 4 pregnancies out of 83 matings and after vitaminization there were 9 pregnancies out of 26 matings ($\chi^2 = 16.75$ and $P = 0.01-$, significant). Of the 4 infertile bucks, 3 became fertile after vitamin treatment whereas before the treatment only one of the 14 matings with known fertile does resulted in pregnancy ($\chi^2 = 8.14$ and $P = 0.01-$, significant). Here it should be pointed out also that 2 does and 1 buck became fertile in matings made as early as 4 days after vitamin treatment began. While such a finding may be incidental, it can not be entirely neglected. There was some degree of improvement, following vitaminization in practically all of the 13 animals grouped under desertion or neglect of the young. Three out of the 6 treated animals grouped under cannibalism raised their young without any attempt to destroy them, while 3 others showed no effect with the first litter following treatment.

Furthermore, prolonged observations on some of the animals reported here indicated that once a correction has been made normal functional behavior may last for some time, even after vitaminization has been discontinued. Infertile animals were found to be fertile on subsequent matings, and animals with tendencies to desert or destroy their young proved to be excellent mothers and raised their own or foster litters without difficulty. On the other hand, some animals returned to their original abnormal state when vitamin treatment was discontinued after a temporary success had been achieved.

Those that failed to respond to vitaminization may have been cases that required much longer treatment than was given, or the abnormality may have been due to factors not correctable by vitaminization. But it is clear that certain cases of abnormal reproductive behavior are associated with a nutritional disorder and that the use of a measure such as vitaminization will correct reproductive abnormalities of this class, although the effect may be general rather than specific. The relative efficacy of the 3 vitamins employed in the experiments was not determined. However, in 16 successfully treated cases yeast alone was used.

7417 C

Gonad-Stimulating Hormone in Urine of a Patient with a Teratoma Testis.*

C. F. FLUHMANN AND P. E. HOFFMANN.

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Zondek,[1] Ferguson,[2] and others have shown that the urine of men with various testicular tumors may contain a gonad-stimulating hormone. Evans *et al.*[3] reported that this substance may have characteristics of both the anterior-pituitary-sex hormone and the factor occurring in the urine of pregnant women. Hamburger[4] found a hormone in the urine of 5 men with testicular tumors and one woman with a teratoma of the ovary, which produced the same histologic picture in the ovaries of immature mice as that resulting from the injection of urine from pregnant women. In a seventh case, a man with an embryoma testis, he obtained results which he felt were due to the presence of 2 hormones. Since the patient had been castrated as a consequence of radium therapy, he believed that one of these substances was due to the presence of the tumor and that the other appeared as a post-castration effect.

The urine examined in this study was from a man 34 years of age, with metastases from a teratoma testis, for which he was under the care of Doctor J. R. Dillon, of the Department of Urology.

A 24-hour specimen of urine contained approximately 2500 rat units per litre. The histologic picture in the ovaries of more than 20 immature rats injected with the urine or with an extract, corresponded to that induced by the gonad-stimulating hormone of pregnancy (Fluhmann[5]) and showed corpora lutea, lutein cysts, and developing follicles. The injection of a known dosage of an extract to 5 immature rats resulted in an average increase in ovarian weight of 190% in 5 days, while the ovaries of 5 littermates given

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* Supported in part by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Zondek, B., *Klin. Wchnschr.*, 1932, **11**, 274.

[2] Ferguson, R. S., *Am. J. Cancer*, 1933, **18**, 269.

[3] Evans, H. M., Simpson, M. E., Austin, P. R., and Ferguson, R. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 21.

[4] Hamburger, C., *Studies on Gonadotropic Hormones*, Levin and Munksgaard, Copenhagen, 1933.

[5] Fluhmann, C. F., *Endocrinology*, 1933, **17**, 550.

the same total dosage over a period of 10 days produced an average increase in ovarian weight of 460%.

Administration of the untreated urine to one hypophysectomized rat, and of an extract to 2 hypophysectomized rats for periods of 7 or 10 days resulted in a very great development of the theca and interstitial tissue. This result is characteristic of the "anterior-pituitary-like" hormone found in pregnant women (Noguchi,[6] Collip *et al.*,[7] Smith and Leonard.[8])

Conclusion. It was found, on the basis of 3 biologic tests, that the gonad-stimulating hormone in the urine of a patient with a teratoma testis had the same characteristics as the substance occurring in the urine of pregnant women.

7418 C

Gonad-Stimulating Hormone in Urine of a Patient with a Chorioepithelioma Uteri.*

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Although the discovery of Rössler,[1] that the urine of women with hydatidiform mole or chorioepithelioma uteri contains large amounts of a gonad-stimulating factor, has been repeatedly confirmed, there is as yet no information available as to the nature of this hormone.

The specimens examined in this study were from a patient under the care of Doctors Reginald Knight Smith and T. Henshaw Kelly. She was a woman of 29, who developed a chorioepithelioma uteri following a hydatidiform mole, and died with extensive metastases in the liver and in the lungs.

A number of extracts were prepared by alcohol precipitation of the urine, and various dosages were injected into more than 25 immature rats. The histologic examination of the ovaries of these

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[6] Noguchi, K., *Jap. J. Med. Sc., Pharmacol.*, 1931, **5**, 104. Quoted by Collip *et al.*[7]

[7] Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

[8] Smith, P. E., and Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1248.

* Supported in part by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Rössler, H., *Z. f. Geburtsh. u. Gynak.*, 1929, **96**, 516.

animals showed the presence of a few corpora lutea, lutein cysts, and developing graafian follicles. The injection of a known dosage of the extract to a group of 5 rats over a period of 5 days produced an average increase in ovarian weight of 63%, while the administration of *the same total dosage* over a period of 10 days to 5 littermates gave an average increase of 90%. It has been shown previously that these 2 findings are characteristic of the gonad-stimulating hormone found in pregnant women and not of anterior pituitary extracts (Fluhmann[2, 3]).

Administration of the untreated urine failed to induce any demonstrable changes in the atrophied ovaries of 2 hypophysectomized rats, but a concentrated extract induced an extensive development of the theca and interstitial tissue in 4 other instances. It has been shown by Noguchi,[4] Collip *et al.*,[5] and Smith and Leonard[6] that this is a characteristic of the gonad-stimulating hormone of pregnant women.

Conclusion. The gonad-stimulating hormone found in the urine of a patient with a chorioepithelioma uteri had certain biologic characteristics similar to those of the substance occurring in women during normal gestation.

7419 C

Accelerated Production of Poliomyelitis.*

JOHN A. TOOMEY.

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When I injected poliomyelitis virus in *Macacus rhesus* monkeys either intrasciatically, intracerebrally or directly into the cord in the region of the first lumbar segment, periods of 4, 7 and 2½ to 3 days, respectively, elapsed before paralysis came on. Such latent inter-

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[2] Fluhmann, C. F., *Endocrinology*, 1933, **17**, 550.

[3] Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1014.

[4] Noguchi, K., *Jap. J. Med. Sc. Pharmacol.*, 1931, **5**, 104. Quoted by Collip *et al.*[5]

[5] Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

[6] Smith, P. E., and Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1248.

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vals are considered the incubation periods of the virus. It is curious that the time elapsing before paralysis appeared was shorter the nearer the injection was made to the lumbar area. Unlike the virus of smallpox, for instance, which multiplies *in situ,* the virus of poliomyelitis spreads away from the cortex and down the cord,[1] practically disappearing from the site of the injection in the cerebrum. It does not cause a great amount of functional damage in the animal until a few days after it reaches the lower part of the cord. In its manner of progress, it acts like a catalyst, a catalyst with nearly an obligate affinity for grey fibers or for axis cylinders of medullated nerves.

In previous experiments, I tried to recover the virus from the stools of humans but failed. However, the stools obtained from monkeys, before and after paralysis, showed a difference in that the feces obtained at the time of paralysis contained a toxic factor that was not present before the onset of paralysis when the animals were in normal condition.[2] The appearance of increased toxicity in the stools of these monkeys coincided somewhat with a marked drop in the agglutinin titer value of their blood serums withdrawn during the stages of paralysis against various enteric organisms.[3]

Because of these findings, my attention was directed to the colon organisms. Could the destruction of nerve cells be due to the effect produced by a combination of poliomyelitis virus and enteric toxin? Such combinations are not new in medicine as Shope[4] has shown in swine influenza and as has been pointed out in Oroya fever.[5] Perhaps the virus needed only the addition of enteric toxins to produce the disease sooner in monkeys. The following experiments were done to test this latter point.

Twenty-three strains of enteric organisms were planted in 0.5% glucose broth, the toxin harvested 10 days later[3] and brought up to a pH of from 7.6 to 7.8.

Nine animals were divided into sets of 3. Each one was injected intracerebrally as noted in Table I. The injecting needle was held in place for a few minutes in order to prevent seepage. All operations were done under complete ether anesthesia. The injection and results are noted in Table I.

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[1] Fairbrother, R. W., and Hurst, E. E., *J. Path. and Bact.*, 1933, **17**, 30.

[2] Toomey, J. A., and vonOettingen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1082; *Am. J. Dis. Child.*, in press.

[3] Toomey, J. A., *J. Inf. Dis.*, 1934, **54**, 74.

[4] Shope, R. E., *J. Exp. Med.*, 1931, **54**, 373.

[5] Oroya Fever, *J. Am. Med. Assn.*, 1933, **100**, 191.

TABLE I.
Intracerebral Injections.

	Positive Control	Negative Control	Virus Toxin Injection
Set I	1.25 cc. 20% Virus plus 1.25 cc. Normal Saline	1.5 cc. Enteric Toxin plus 1.5 cc. Normal Saline	1.25 cc. Enteric Toxin plus 1.25 cc. 20% Virus
Monkey and Result 6 lb. Animals	Animal A—paresis on the 7th day and quadriplegia on the 9th day.	Animal B—no reaction.	Animal C—hemiparesis in 24 hours, paralysis the 3rd day and quadriplegia the 5th day.
Set II	1.5 cc. 10% Virus plus 1.5 cc. Normal Saline	1.5 cc. Enteric Toxin plus 1.5 cc. Normal Saline	1.5 cc. Enteric Toxin plus 1.5 cc. 10% Virus
Monkey and Result 6 lb. Animals	Animal D—paresis the 9th day and paralysis the 11th day. Animal lived.	Animal E had no reaction.	Animal F—hemiparesis in 24 hours, paralysis the 5th day and quadriplegia the 6th day. Animal died.
Set III	1 cc. 10% Virus plus 1 cc. Normal Saline	1 cc. Enteric Toxin plus 1 cc. Normal Saline	1 cc. Enteric Toxin plus 1 cc. 10% Virus
Monkey and Result 4½ lb. Animals	Animal G—paresis the 8th day, paralysis the 10th day, quadriplegia the 13th day.	Animal H had no reaction.	Animal I—hemiparesis in 24 hours, paresis of the right side and left foot the 5th day and quadriplegia the 7th day.

After abdominal section, combinations of enteric toxin and virus which were introduced into the small intestine in an area isolated between 2 intestinal clamps produced the disease quicker than when only virus suspensions were used. Enteric toxin injections alone had no effect.

After abdominal section, combinations of enteric toxin and virus which were injected into the subserosa of the small intestine (25 cc. of 2% virus and 25 cc. of enteric toxin) produced the disease more quickly than when only virus suspensions were used (50 cc. of 1% virus). Enteric toxin injections alone had no effect (25 cc. of enteric toxin and 25 cc. of saline).

The enteric toxin, obtained after growing the ordinary colon bacilli found in the gastrointestinal tract for 10 days in glucose broth, when combined with poliomyelitis virus, accelerated the production of poliomyelitis in the *Macacus rhesus* monkey.

7420 C

Fibrinolysis by Streptococci of Human and Animal Origin.*

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The discovery by Tillett and Garner[1] of an antihuman fibrinolytic function in *S. hemolyticus* isolated from human cases and the absence of this enzyme from apparently identical streptococci isolated from the environment or from lower animals, is of suggestive epidemiologic interest. We have, therefore, attempted to confirm their findings.

To do this, 193 cultures of typical hemolytic streptococci were kindly furnished by various clinics and veterinary institutions of Northern California. Antihuman fibrinolytic tests were made by the Tillett-Garner technic, each test being repeated 3 times with different samples of normal human blood. Control tests were made with stock cultures of known lytic powers.

For convenience in recording our data, the 193 cultures were divided into 3 groups: (a) 32 cultures originally isolated from internal human tissues, representing such diseases as pneumonia, septicemia, empyemia, endocarditis, osteomyelitis, and meningitis; (b)

TABLE I.

Lysis of Normal Human Plasma-Clot by 24 Hour Broth Cultures.

Composite readings from 3 tests with different samples of normal human blood; ++++, complete liquefaction of plasma-clot by the end of 60 minutes; +++, complete liquefaction by end of 2 hours; ++, practically complete lysis in from 6 to 24 hours; +, partial lysis by end of 24 hours. Cultures were considered negative which gave no demonstrable softening of the clot by the end of 24 hours.

S. hemolyticus isolated from:	No. of cultures tested	No. giving fibrinolysis				No. not fibrinolytic	Lytic %
		++++	+++	++	+		
(a) Internal human tissues	32	14	7	8	1	2*	94
(b) Superficial human tissues	123	6†	7†	7	1	102	17
(c) Veterinary tissues	38	0	0	0	3‡	35	7

*One of these negative strains was from a right mastoiditis; left mastoiditis, same patient, 10 days later + + + lysis. The other negative strain was from acute meningitis.

†This includes 5 erysipelas strains, all of which were strongly lytic.

‡The 3 slightly positive strains were from hog, dog, and deer.

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* Supported by CWA. (Discontinued April 1, 1934.)

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

123 typical hemolytic strains originally isolated from superficial human tissues, representing such diseases as erysipelas, furunculosis, and fistula, septic sore throat, sinusitis, and acute gastric disturbance; and (c) 38 cultures of veterinary origin, representing such animals as cow, horse, hog, dog, deer, and rabbit. The lytic tests thus grouped are recorded in Table I.

Groups I and III are in practical accord with the Tillett-Garner data. Group II, however, suggests a much less sharp differentiation between antihuman and antiveterinary streptococci than their data indicate. The low percentage of fibrinolytic hemolytic streptococci in this group suggests that a fibrinolytic typing of superficial human infections might be of clinical interest.

Thirty-three strains of *S. viridans* were tested in connection with this work. All were non-fibrinolytic.

7421 P

Lactose Degraded Colon-Aerogenes Organisms in Normal Feces.

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The type of degradation of lactose-fermentation here considered is that in which bacterial growth in lactose broth produces no fermentation the first few days of incubation. After 2 to 14 days, acidity and gas production may appear. In other strains it is only after repeated subculture on lactose that this sugar is fermented. As a result of lactose-fermenting deficiency, degraded organisms are entirely missed in water analysis. Since dextrose fermentation is normal, degraded strains grow on Endo and Russell's medium like paratyphoids and complicate the examination of suspect material for members of the Salmonella group. Many, but not all, strains ferment saccharose promptly. This led Krumwiede[1] to add saccharose to Russell's medium to exclude these degraded forms designated in Park and Williams as "paratyphoid-like intermediates". Kennedy *et al.*[2] have presented data for 22 strains of colon bacilli showing delayed lactose fermentation, in urine, stools and water.

We have been interested in the *Escherichia-Citrobacter-Aerobac-*

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[1] Krumwiede, Charles, and Kohn, L. A., *J. Med. Res.*, 1917, N. S. **32**, 225.

[2] Kennedy, J. A., *et al.*, *J. Infect. Dis.*, 1932, **50**, 333.

ter ratio in saline suspensions of feces, fresh and after storage at 37° and icebox temperature. Lactose degraded organisms have been encountered. The fecal suspensions were first plated on Endo from which representatives of all colony types were fished to dextrose broth. From these tubes plates were again made on Endo and a well isolated colony picked to lactose broth. From this growth a third Endo plate was made from which again a well isolated colony was fished to lactose. By such technique we were reasonably assured of pure cultures and were able to exclude growth which failed to ferment dextrose. The strains isolated were tested for their gelatin liquefaction, action on litmus milk, growth on Simmons' citrate agar, production of indole and hydrogen sulfide, their methyl red and Voges-Proskauer reactions and for fermentation of saccharose, dulcite, cellobiose, α-methyl-d-glucoside and Levine's boric acid lactose broth.

In this manner 27 different fresh fecal suspensions yielding 306 strains were studied. Three of the original suspensions were kept at 37° until no more colon-aerogenes organisms could be recovered (60, 73, and 77 days respectively), during which time 303 strains were recovered from 47 platings. Six original fresh fecal suspensions, of which 3 duplicate those kept at 37°, have been stored in the icebox and have yielded 449 strains on 80 platings. In no one of these icebox suspensions have all colon-aerogenes yet disappeared (longest interval 173 days).

One lactose degraded organism *(Aerobacter)* was encountered among the 306 from fresh feces, 45 (35 *Aerobacter*) among 303 strains from suspensions stored at 37° and 82 (78 *Aerobacter*) among 449 strains from suspensions in the icebox. Nine of the 128 degraded strains were indole-negative *Escherichia* and of these 8 were from suspensions stored at body temperature. Three were *Citrobacter* and 2 did not fall into our commonly accepted classifications.

Lactose degraded colon-aerogenes organisms are not numerous in freshly excreted feces but increase greatly in numbers when fecal suspensions are stored. That this deficiency is due to a degradation of ordinarily normal organisms seems likely. To test this point pure cultures of typical Escherichia and Aerobacter were inoculated into flasks of sterilized fecal suspension and stored. The lactose fermenting capacity of the *Escherichia* has been but slightly influenced thus far but it has been possible to reduce the *Aerobacter* to a typical slow lactose fermenter. At the same time its dextrose, saccharose and cellobiose fermentations have not been affected.

Furthermore the degraded strains encountered have in many cases been reverted to typical lactose fermentation.

The lactose deficient Coli previously described by many workers probably represents a type of organism degraded by processes more significant than those ordinarily occurring in the normal bowel or in simple storage. Bacteriologists should know that types apparently *Salmonella* deserve careful study including serological indentification before being designated paratyphoids. This is particularly true in examinations of suspected carriers or of materials concerned in food poisoning. The value of the Krumwiede modification of Russell's double sugar medium as an aid in eliminating saccharose fermenting, lactose degraded organisms of this group has been confirmed but it must be remembered that a certain number of these deficient organisms will not ferment saccharose (14 of Kennedy's 22, for example) and hence would still pass culturally as *Salmonella.* The desirability of holding lactose broth cultures for several days when negative results are apparently obtained or indeed inoculating such tubes in series day by day, should be borne in mind. It is striking that the fermentation of lactose, the carbohydrate of prime importance in this group, should be the function most subject to degradation and that *Aerogenes,* an organism of reputedly strong fermentative power should appear as the predominant degraded organism under normal conditions.

7422 P

Bacteriological Observations on a Case of Meningo-Encephalitis.*

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On December 12, 1933, a nurse became suddenly ill and repeatedly vomited bile stained material. The following morning she had a severe chill, a temperature of 101°F., severe headache, some rigidity of the neck and a doubtful Kernig's sign. A day later her temperature reached 105.3°F. and the meningeal symptoms were more marked. During the first 10 days of her illness the more prominent symptoms were: fever, headache, occipital tenderness, rigidity of the neck, strabismus, ptosis of left eyelid, diplopia, twitching muscle fasciculi and lethargy from which she could be fairly easily roused. There was gradual improvement after about the tenth day. Ten weeks after the onset there remained some speech defect, difficulty in feeding herself, dysmetria, sprawling gait, slight nodding of head, emotional instability and impaired memory.

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* The experimental studies here reported were supported by the Mrs. Mary Hooper Somers Medical Research Fund.

Spinal fluid obtained December 15 was hazy and contained 812 cells per cu. mm. (95% small lymphocytes). The sugar content was 37 mg. per 100 cc. and the gold curve 0023321111. Subsequent specimens gave a paretic curve. Repeated Wassermann tests have been negative. Inoculated guinea pigs failed to develop tuberculosis.

Of special interest is the fact that an apparently new Gram-positive bacillus was obtained in pure culture from the spinal fluid on December 18, 19, 21, 22, 26, 28, 1933; January 3, 8, 10, 18, March 8, April 12, 1934. Cultures made May 10 were negative. A rabbit inoculated intracerebrally with 0.5 cc. of the spinal fluid obtained on December 28 developed a severe encephalitis 2 days after inoculation. The same organism was cultivated from the brain tissue of this animal.

In the original broth and blood agar cultures the organism is a short, plump, bacillus (0.4-0.6×0.6-$1.0\ \mu$) with rounded ends, which on subculture on certain media becomes longer (1-2 μ). In Veillon agar stabs, incubated at 37°C., it tends to give rise, even in young cultures, to much longer rods (2-10 μ), sometimes mixed with straight and spiriliform threads (15-20 μ), short comma forms, ring forms and solid spherical bodies. These forms revert promptly to short, plump rods on transplantation to broth or other media. It is Gram-positive, but easily decolorized, non-motile, does not form spores, is not acid-fast and not capsulated. What seem to be small metachromatic granules and polar staining may occasionally be observed in suitably stained larger forms. It is a facultative anaerobe, grows well on the usual laboratory media and is not exacting in its temperature requirements. On blood agar plates, it forms small streptococcus-like colonies which may in several days reach a diameter of about 1 mm. After 48 hours a well defined zone of almost complete hemolysis is present. Growth on blood agar slants is delicate and resembles that of hemolytic streptococcus. On glycerine agar growth is more evident and whitish in color. A faint uniform turbidity is imparted to liquid media. It ferments the following carbohydrates without gas production: dextrose, levulose, rhamnose, xylose, maltose, dextrin salicin, and adonitol. Lactose, sucrose and glycerol are fermented slightly after some days. It does not ferment galactose, raffinose, inulin, erythrol, mannitol nor dulcitol. Neither does it liquefy gelatin nor coagulated blood serum. Hydrogen sulphide and indole are not formed. Litmus and methylene blue are strongly reduced, but nitrates are not.

The organism is pathogenic for mice, guinea pigs, rabbits and rhesus monkeys, freshly isolated cultures generally being fatal within 72 hours after intravenous or intracerebral inoculation. Evidence of meningeal irritation and incoordination is frequently prominent. Rabbits seem most susceptible. The most characteristic gross lesions are minute (0.5-1 mm.), yellowish, opaque miliary lesions which may be located in the meninges, brain, kidney, adrenal, heart muscle, spleen and liver. The adrenal may be markedly congested. Microscopically there are small foci of necrosis, surrounded and invaded by proliferating histiocytes and leucocytes and frequently interstitial hemorrhage. In the central nervous system, the lesions are found particularly in the leptomeninges and the adjacent nervous tissue. The organisms are easily cultivated from infected tissues.

Further studies on the properties of the organisms are in progress. The evidence at hand indicates that systematically it falls in the so-called diphtheroid group. We suggest for it the name *Corynebacterium parvulum.*

7423 P

Phosphatase Activity of the Serum and Tissues in Osteogenesis Imperfecta.

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Robison[1] has pointed out that the enzyme phosphatase is an important factor in the calcification of bone. Kay[2] has more recently summarized the evidence for its activity in connection with studies on a wide variety of clinical conditions. Since osteogenesis imperfecta is an outstanding example of defective bone formation for which no pathogenesis has as yet been discovered, a comprehensive study of the various metabolic factors which might be involved in this condition was begun by the writer in 1931. The present report deals with the phase of that study which concerns the phosphatase activity of the blood serum and fixed tissues.

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[1] Robison, R., *Biochem. J.*, 1923, **17**, 286.

[2] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 235, 249.

Kay reported the serum phosphatase activity to be slightly increased in some cases of osteogenesis imperfecta, while Bodansky and Jaffe[3] found it to be normal in 6 cases.

Periodic determinations of the serum phosphatase by the method of Kay have now been made in 4 cases of osteogenesis imperfecta under a variety of experimental conditions. In one case the fixed tissue phosphatase has also been studied postmortem.

Repeated determinations in the 4 cases showed values almost identical with those found for normal control subjects and for a miscellaneous group of patients in the same age period. Variations in the ordinary constituents of the diet were found to have no definite effect on the serum phosphatase values. Following the administration of vitamin D in relatively enormous doses in the form of viosterol 10,000X, however, the phosphatase activity of the serum was uniformly reduced. Parathormone administration over a period of several days had a similar but less marked depressing effect on the enzyme.

The sudden death of a 7-year-old girl with osteogenesis imperfecta, at a time remote from any unusual medication and when no demonstrable infection was present, made possible a study of the phosphatase activity of the blood serum and fixed tissues a few hours postmortem. The serum values were normal. Figures found for the various tissues were as follows:

Tissue	Units Phosphatase*
Liver	0.12
Muscle	0.17
Brain	0.14
Kidney	0.28
Intestine	
Duodenum	0.00
Jejunum	0.09
Ileum	0.40
Cecum	0.21
Colon	0.39
Bone	
Ulna, upper end	0.83
Ulna, lower end	0.50
Ulna, shaft	0.65
Periosteum and subperiosteum	0.00
Rib	0.76
Callus rib	1.05

* Expressed in mg. phosphorus liberated by 1 gm. of tissue in 3 hours at 38°C. and at pH 8.8.

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[3] Bodansky, A., Jaffe, H. L., *Proc. Am. Soc. Biol. Chemists,* 1934, 11.

The most striking feature of the results was the almost complete absence of phosphatase in the periosteum and sub-periosteal structures, where it is normally abundant. This observation suggests a relationship between the extremely defective calcification of the cortex and the lack of phosphatase. That phosphatase may be mobilized from other sources under special conditions, however, is indicated by the greatly increased activity in the region of callus formation.

The extremely low phosphatase activity found in the duodenum may be of significance in relationship to defective phosphorus absorption. Normally the duodenum shows a higher phosphatase activity than other segments of the gut.

7424 P

Prophylactic Vaccination Against Intracranial Complications Following Pneumococcus Type III Mastoiditis.

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Inasmuch as intracranial complications following pneumococcus Type III mastoiditis are known to occur, as a rule, not earlier than several weeks after the initial infection, serious attempts to induce a state of active acquired immunity should receive special consideration. The use of prophylactic vaccination was suggested by Kolmer and Amano on the basis of their work in rabbits in which immunization with pneumococcus vaccines elicited protection against experimental meningitis.[1] Barach found that type-specific active acquired immunity appeared within 6 days after intradermal or intravenous injection of pneumococcus type-heterologous vaccines in cases of lobar pneumonia.[2]

Accordingly, in September, 1931, we undertook to immunize every case of pneumococcus Type III mastoiditis admitted to the Otological service of The Mount Sinai Hospital as soon as the bacteriological diagnosis was made, whether that be before or after the operation. The following method of vaccination was employed:

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[1] Kolmer, J. A., and Amano, K., *Arch. Otolaryngology*, 1931, **14**, 125; 1932, **15**, 547.

[2] Barach, A. L., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 558; *J. Exp. Med.*, 1931, **53**, 567.

The patients were given a course of 6 intradermal injections, 2 a week, in the following increasing doses: 0.1 cc., 0.2 cc., 0.3 cc., 0.5 cc., 0.7 cc., and 1 cc. For doses larger than 0.2 cc. several simultaneous injections of 0.2 cc. each were made. In order to avoid loss of time, a stock vaccine was used for the initial injection. This was done never later than 48 hours after the operation and sometimes before the operation. For the remaining 5 injections an autogenous vaccine was used. The autogenous vaccine was prepared by growing a pure culture of pneumococcus Type III in 100 cc. of 1% glucose broth for 24 hours. The organisms were centrifuged, washed once and then resuspended in 0.85% NaCl solution to obtain a concentration of six hundred million organisms per cc. The bacteria were then killed by heating at 60° for one-half hour.

Twenty-seven cases of pneumococcus Type III mastoiditis have been admitted to this hospital since September, 1931. Of these 2 died within 48 hours after operation and since they received either no vaccination or only one injection the day before death, they will not be considered in this series. Of the 25 patients receiving the vaccine-therapy, one patient promptly recovered but 4 months later developed meningitis and died. The remaining 24 cases made uneventful recoveries. Thus, the mortality rate of our vaccinated series was 4%.

During a period of 5 years (1927 to 1931) prior to this work, 547 patients suffering from mastoiditis were admitted to this hospital. Of these, 525 suffered from non-pneumococcus (streptococcus hemolyticus, etc.) mastoiditis and 22 from pneumococcus Type III mastoiditis. Of these twenty-two, 5 died of meningitis, thus forming a mortality rate of 22%.

We are well aware of the fact that our cases constitute too small a group from which to draw definite conclusions; that the pneumococcus Type III mastoiditis might have been of a milder type during the past 3 years; and the lower incidence of cerebral complications in our vaccinated series might have been merely a fortunate coincidence. However, we feel that the facts presented warrant a preliminary report in order to stimulate further work along these lines.

We are greatly indebted to the Otologist of The Mount Sinai Hospital, Dr. I. Friesner, for his generous cooperation and interest in this problem.